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polymerase chain reaction (PCR) and its various modifications have added new dimension by facilitating the overall genome analysis. This encompasses the individualization of genome to assess variation within the species, genetic structure of the populations, reproductive behaviour of animals, phylogenetic relationship of desired clads[2]. DNA sequences originate and undergo evolutionary metamorphosis and may to be used as powerful genetic markers in a wide range of species. Detailed studies have been carried out in domestic species like cattle[3], goat[4], sheep[5], pig[6], poultry[7] and dogs[8] but negligible attempts have been made to undertake molecular characterization of bubaline genome in the context of breed delineation, genome individualization and phylogenetic positioning. We have studied several variable repeat loci in the bubaline genome using DNA markers reported from other species and have also cloned and characterized a satellite fraction following established approaches[9,10]. DNA typing from blood and semen samples of the same males was found to show sequence modulation in the meiocytes, which is restored in the somatic cells" (Chattopadhyay *et al.* 2001). Similarly, a genome derived cloned proble pDS5 (GenBank Accession No. Y-07658) representing α collagen gene seems to be tagged with satellite DNA having about two thousand copies in the bublaine genome[12]. Assessment of other structural or regulatory genes tagged with satellite DNA will provide a useful tool to follow the segregation pattern of the desired gene and their possible modulation in different population within the same species. Clearly, additional work is required to prove or otherwise these conceptual dogmas in the context of animal biotechnology. Thus, studies on repetitive DNA, development of genetic marker and direct sequence analysis of desired loci including that of mt genome of the bubaline will provide better understanding. In this context, overall organization of several species of repetitive DNA, its origin and interspecies variation, leading to the development of genetic markers and relevance of studying mt genome are discussed. In addition, applications and implications of PCR technology facilitating several aspects of genome analysis are highlighted.

1. Genomic Organization of Repetitive DNA

A significant percentage (about 30-40%) of all mammalian genomes consists of repeate DNA[13] whose origin, evolution and functions are still being actively pursued to understand their biological significance. These sequences are arranged either as tandem arrays or interspersed in the genome[14]. Tandemly arrayed repeats include highly variable minisatellite and microsatellite regions comprising repeat units of about 7 bases or more[15]. Interspersed repeated DNAs include short interspersed elements (SINEs) and long interspersed elements (LINEs)[16]. These sequences have been hypothesized to originate from functional genes through a retroposon like mechanism. The sequences are spread in the genome by several

rounds of transcription and reverse transcription by RNA polymerase III to generate SINEs and RNA polymerase II to generate LINEs[17].

Majority of minisatellites/microsatellites composed of short repeats of 2-10 bp is interspersed within the coding sequences and conserved in eukaryotes. However, their genomic organisation varies even within the same species[18]. Further, evolutionary forces acting upon these repeat loci change their copy number by localized amplifications or by insertion of tandem repeats, generating hypervariability in the genome[19]. Several other mechanisms such as strand slippage during DNA replication, base misalignment and unequal crossover between homologous chromosomes during meiosis and sister chromatid exchanges leading to organizational uniqueness of the species have been proposed[20]. Though, not proven always for each eukaryotic genome, insertion of viral sequences altering the organizational integrity of the genome has been reported[21-23]. Thus strategies need to be evolved to uncover organizational variability of the genome encompassing all these aspects.

Evaluation and inter-species variation of repeat sequences in the population

As mentioned earlier, mechanisms of loss or gain of repeat by unequal crossing over and gene conversion can lead to homogenization (molecular drive) of any given variant in a sexual population[24]. During the evolution of minisatellites by unequal crossing over, some variants will be lost whereas other will increase in frequency, eventually replacing all others. Such genetic drift in the alleles of a repeat array will purge all the point mutations. These evolutionary changes occurring in the minisatellites lead to homogeneity in the repeats of an array within a species but heterogeneity in the units of the corresponding array in different species giving rise to inter-species variations[25]. However, this phenomenon will be affected by the male female ratios, overall population size and possibility of infusion of newer genetic materials in a given gene pool and allele fixation will involve evolutionary incubation of time[2].

Functional significance of repeat sequences

Repeat sequences have been implicated in gene regulation[26], replication of telomeres[27], selection and transport of mRNA molecules to the cell cytoplasm[28] and as signals for recombination and gene conversion[29]. A number of probes based on repeat sequences with varying length and base composition have been used successfully as genetic markers[30-33].

2. Genesis of Genetic Markers for BubalineGenome

Earlier, conventional protein and biochemical markers were used for breeding program of bubaline species[34]. Subsequently, these were replaced by diallelic RELP for the loci homologous to cattle[35]. However, owing to low level of polymorphism detected with these markers, their application remained rather limited[36]. Thus far, only two homologous loci

have been characterized in buffalo compared to a total of 240, 73 and 8 loci in cattle, sheep and goat respectively[37]. Non-availability of informative genetic markers has also hampered cross breeding programme of this species. With the discovery of hypervariable repeat loci[38] and their use as polymorphic probes in several eukaryotes particularly for DNA fingerprinting[18,20], it has become possible to uncover overall genetic variabilities of the bubaline genome and establish linkage of the same with its physical and physiological attributes. DNA probes based on synthetic oligonucleotides have been reported to be equally informative for DNA fingerprinting. While probes based on repetitive DNA have been found to be informative for bubaline and other related genomes[9], no clear cut and well defined experimental approach is documented that could assist the identification and segregation of the elite animals with distinctly superior QTL loci. This is due to the fact that most of the physical and physiological attributes recognized to be part of the elite animals, are controlled by several genes (polygenic characters) and it is extremely challenging to lay hand on all such genes implicated in conferring the superior traits. Nonetheless, there is a growing consensus that if an animal genome were saturated with sufficient markers encompassing all the genes, that would positively assist the identification of QTL loci. Identification of Fecundity gene in sheep is one such convincing proposition[39]. Following the segregation of the genes tagged with satellite DNA may prove to be one of the useful approaches in this direction[11].

Oligonucleotide based probes for DNA fingerprinting

DNA fingerprinting was first demonstrated by genome derived cloned probes[20]. Soon after, synthetic oligo probes for genome individualization were reported[40]. Oligonucleotide probes are absolutely specific owing to formation of perfectly matched duplexes with target DNA because mismatched bases are dissociated during hybridization[41]. Optimization of oligo probe length for identifying maximum number of variant alleles was first attempted using different repeat unit length of $(GATA/GACA)_n$ in the human genome[40]. In subsequent studies, additional probes of 2-6 base repeat motifs such as $(AT)_n$, $(CA)_n$, $(GAA)_n$, $(TCC)_n$, $(GACA)_n$, $(GATA)_n$, $(GGAT)_n$, $(GGCA)_n$ and $(TTAGGG)_n$ were reported[18-42]. The number of different oligo probes reported to be useful for different eukaryotic genomes studied so far is about 20 or less[13]. Additional informative probes useful for DNAF may be developed by undertaking characterization of individual hypervariable repeat loci from desired species followed by the search and development of oligo probes based on core or consensus element of the repeat arrays.

Somatic and germline mutation and its implication

Somatic instabilities of the repeat sequence have been reported in several genetic disorders like myotonic dystrophy, fragile X syndrome[42], Kennedy's syndrome,

Huntington's disease and in tumor DNA[43,44]. This includes chromosomal deletion, mitotic non- disjunction and recombination[46,47] leading to loss of associated minisatellites[48]. Tissue or tumor-specific changes owing to DNA methylation affecting band profile(s) have also been reported[48]. In humans, a gene involved in DNA repair located on chromosome 2 has been suggested to be causing instability to mono-, di- and trinucleotide repeates in colon cancer[49]. Whatever be the mechanism, mutation rate of the hypervariable loci, used as markers for DNA profiling has great repercussions in forensic cases and paternity testing[51]. Parentage exclusions with limited number of hypervariable loci would lead to false exlusions of genuine parents if the mutation rates were significantly higher[52]. Mutation rates of approximately 10^{-2} per gamete do not drastically interfere with the use of these probes in paternity analysis, provided their rate of occurrence is known and can be incorporated into the statistical likelihood ratio analysis[53]. Germline mutations will produce apparent exclusions in paternity testing whereas somatic mutations would give rise to divergent DNA fingerprint pattern from different tissues of the same individual. Thus, it is critical to study mutation rate in different tissues before using a probe for actual DNA profiling of a species.

3. Development of Methodologies

A number of experimental methodologies used for DNA profiling have been developed and some standard ones are given here.

DNA Isolation and Southern blot analysis

Blood samples of buffalo and other experimental animals such as horse, rabbit, pig, cattle, goat and sheep belonging to both sexes were used for DNA isolation following standard protocols[40]. Digestion of DNA with restriction enzyme(s), (*Alu*I, *Hinf*I, *Hae*III, *Mbo*I, *BamH*I, *Hind*III, *Msp*I, *Pst*I, *Puu*II, *Taq*I, *Xba*I, *Ava*III, *Cla*I, *Dra*I, *Eco*RI and *Kpn*I), was conducted following supplier's specifications (NEB, USA). Electrophoretic separation of DNA, its transfer on the Nylon membrane (Sartorius) and immobilization by exposure to UV was carried out as reported earlier[10]. During bubaline and related genome analysis, we used a set of seven synthetic probes and a commercially available poly dC-dA. The origin and rationale of using these probes and their detail hybridization polymorphism has been reported earlier[9].

Radiolabelling of oligo probes

Oligos were purchased from Biosynthesis Inc. USA and poly dC-dA was obtained from Pharmacia Biotech, USA. Oligo probes were labelled with [γ^{32}P]-dATP (Amersham, UK) and poly dC-dA by nick translation (Amersham, UK) using [α^{32}P]-dCTP (Amersham,

UK). The hybridizations, post-hybridization treatment and autoradiography were carried out following standard protocols[40].

Cloning of a repeat fraction from the bubaline genome

In an attempt to gain an insight into the organization and allele length variation of a satellite fraction from bubaline genome, tensive restriction survey with enzymes mentioned earlier was conducted[10]. We identified a *Bam*HI repeat fraction of about 1.3 kb that was cloned, sequenced and sequences were deposited in the GenBank vide accession No. Y-07658. Following this, we used clone pDS5 as probe to study its expression in germline and different somatic tissues of the bubaline genome. We also identified missing alleles during the analysis of allele length variation using different enzymes and pDS5 probe with DNA from semen and blood samples of the same bubaline males.

Molecular characterization of the bubaline genome with pDS5 recombinant clone

The clone (pDS5 cross-hybridized with cattle, goat, sheep and gaur genomic DNA but not with samples from non-bovid or invertebrate sources indicating that the same is not conserved evolutionarily. Approximately, 2000 copies of pDS5 are estimated to be present in the bubaline genome[12]. The pDS5 showed multilocus monomorphic bands in combination with a number of restriction enzymes in buffalo (Fig. 1) and uncovered allele length and copy number variations amongst related bovids (Fig. 2). Expression studies based on RNA slot blot, Northern blot and RT-PCR amplification showed that barring heart, pDS5 is transcribed in most of the buffalo somatic tissues, testes and ovary[11]. Database search revealed high level of homology of pDS5 with collagen gene VII[53]. Our work suggests that pDS5 is a transcribing satellite, conserved uniquely in the bubaline but organized differently in the related bovid genomes. Interestingly, typing of buffalo blood and semen samples from the same male animal showed allele dropout with respect to two bands (not shown). This suggests that sequence modulation within pDS5 arrays takes place during spermatogenesis and the same is restored after fertilization. The pDS5 may be used for typing semen and blood DNA samples from other bovids to substantiate or otherwise if the sequence modulation is a common phenomenon in related genomes as well or the same is confined to bubaline only.

Synthetic oligo probes detect polymorphic loci in the bubaline genome

Of all the probes used for buffalo DNA profiling, GATA/GACA were not informative whereas poly dC-dA (hereafter referred to as poly CA) with *M*bol detected low level of polymorphism with probability of identify 6.2×10^{-4} amongst unrelated bubaline DNA samples. Similarly, with $(GGAT)_4$ and *Hin*fI enzyme the same was found to be 7.05×10^{-4} and the mean allelic frequency was calculated to be 0.1842. Oligo probes $(TGG)_5$, $(TGG)_6$,

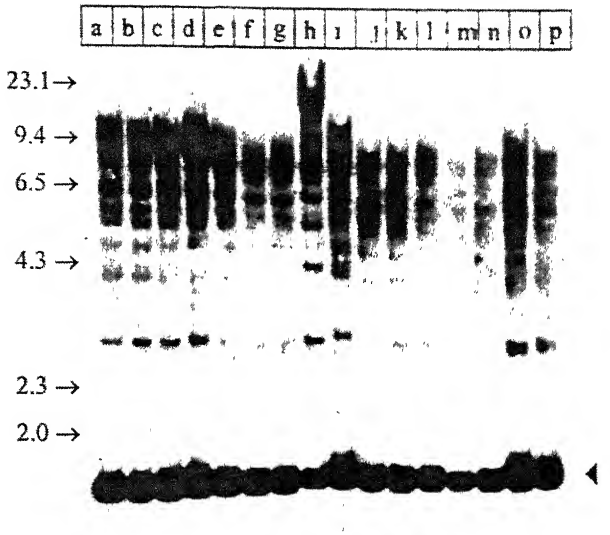

Fig. 1

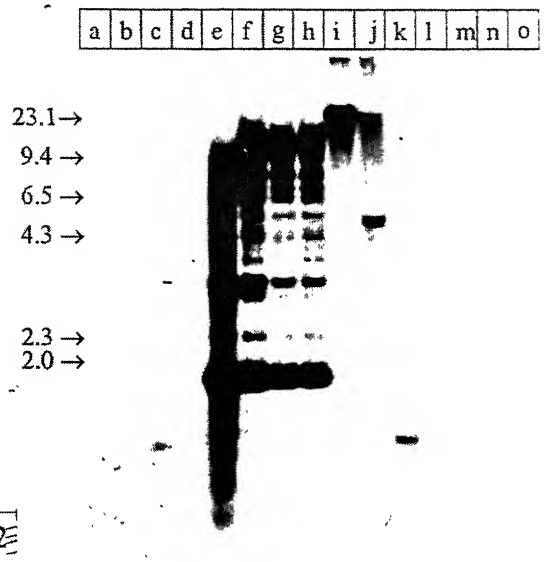

Fig. 2

Fig. 1 – Typing of different breeds of buffaloes genomic DNA digested with *Bam*HI and hybridized with pDS5 probe. Note the multilocus monomorphic bands in all the samples and absence of discermible allele length and copy number variation. Note also the 1.3 kb distinct band (arrow). Lanes a-d represent Toda breed, e-h Mehsana, i-I Surti and m-p Murrah. Stronger signals seen in samples o and p are owing to more amount of DNA used therein. Molecular size marker λ *Hind*III is given in kb.

Fig. 2 – Species-specific band profiles uncovered by pDS5 in bovids with *Bam*HI enzyme. Lanes a-d represent goat, e-h buffalo, i-j cattle, k-I sheep, m-n pig and o human DNA samples. Note the allele length and copy number variation amongst different species showing strong signals in buffalo and weak ones in goat samples. Smear in lane e having buffalo DNA is owing to more quantity of the same loaded therein. Owing to undigested cattle DNA in lane i, signal remained confined only in the high molecular weight region. Note absence of signals in pig and human DNA samples. Molecular size marker λ *Hind*III is given in kb.

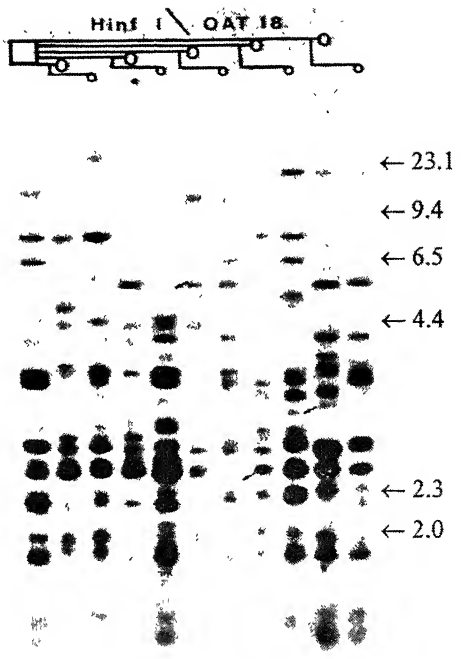

Fig. 3

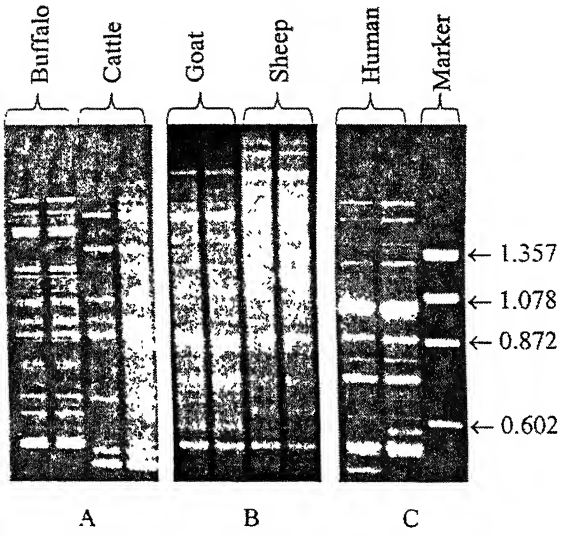

Fig. 4

Fig. 3 – The DNA profile of a Murrah half-sib family detected by the OAT18/*Hin*fI probe enzyme combination. The arrows indicate mutant bands in the offsprings that are not present in either of the parents. The mutation rate detected herein remain within the acceptable limit and do not impinge upon the overall low level of probability of identity critical for genome individualization of this species. Molecular size marker λ *Hin*dIII is given in kb.

Fig. 4 – Minisatellite associated sequence amplification (MASA) with genomic DNA from buffalo, cattle, goat, sheep and human using primer representing consensus sequences (5' CACCTCTCCACCTGCC 3') of 33.15 repeat loci. The amplicons in the range of 2.5 kb to 200 base pairs were resolved on 2% agarose gel using 0.5X TBE buffer. Despite morphological similarity between buffalo and cattle (A) and goat and sheep (B), the band profile is unique for all the species including human. Note the multiple bands, nonetheless distinct species-specific pattern. MASA has an applied potential for unequivocal species identification in the wildlife conservation program in the context of ever growing menace of poaching and human identification from the trace amount of biological sample in the context of forensic medicine.

$(TGG)_7$ (hereafter referred to as OAT15, OAT18 and OAT21 respectively) were found to be most informative with *AluI, HinfI, HaeIII* and *MboI* and detected maximum number of polymorphic bands (Fig. 3). Employing standard methods[54,55], polymorphic information content (PIC) of each probe/enzyme combination on random and family samples were established. The OAT18 probe in combination with *HinfI* enzyme showed maximum polymorphism and the probability of any two random individuals having an identical band profile was calculated to be 2.08×10^{-11}. The overall mean allelic heterozygosity detected in Murrah and Surti breeds with OAT18 probe even in the family samples was found to be very high (~95%). Of the three versions of $(TGG)_n$ used as probes, the maximum number of resolvable bands were detected by OAT15 whereas the maximum number of variable bands were detected by OAT18. This suggests that OAT18 is an appropriate probe for DNA fingerprinting of the buffalo genome[9].

Mutation rate and reliability of the DNA fingerprinting probe

The estimation of mutation rate is important to ascertain germline stability of variable loci. If the occurrence of the mutant alleles (bands) is significantly high, the chances of appearance of novel alleles that cannot be traced back to either of the parents will also be high[9]. This would defeat the whole purpose of using a probe for genome individualization or for resolving paternity disputes. The observed frequency (0.03) of mutant alleles in the bubaline genome scored by OAT18 is significantly lower than that reported earlier (0.07) in cattle using a panel of six microsatellite probes[34]. Thus, OAT18 probe exhibits sufficient germline stability fulfilling the prerequisite condition for DNA fingerprint analysis. However, it does not mean that other probes useful for buffalo DNAF may not be developed. In subsequent studies, we reported other synthetic oligo probe that we used for actual farm condition[56].

Scoring of DNA bands for statistical calculation and phylogenetic analysis

For statistical analysis, conventional estimates of probabilities for identity were followed[8,30,53-55]. The chance that another unrelated bull is the sire of a calf was calculated by paternity index[55,57]. Phylogenetic analysis of the data was carried out using Sequential, Agglomerative, Hierarchial and Nested (SAHN) clustering method in the NTSYS-pc (version 1.70) programme[58]. Each band was scored as binary codes, for the presence "1" or absence "0 in the Operational Taxonomic Units (OTUS) and a similarity matrix (using Jaccard coefficient) and subsequently a phenogram via the Unweighted Pair Group average Method with Arithmetic averages (UPGMA) was generated. Our analysis showed that bubaline is closer to *Ovis aries* (sheep) than cattle despite morphological similarities with the latter. The DNA profiling of all the four breeds with OAT18 and *HinfI* combination revealed high level of heterozygosity in Toda and Mahsana as compared to that of Surti

and Murrah breeds[9]. The heterozygosity detected was 96.2% in Mehsana, 96% in Toda, 93.6% in Surti and 92.5% in Murrah breeds[9].

Significance of breed affiliation

Of the four breeds studied, the Toda resembled swamp buffalo in its phenotypic appearance but showed similarities with Murrah in terms of karyological pattern[60]. The phylogenetic relationship of Toda, based on sequence variability detected at microsatellite loci showed that sequence divergence in this breed is significantly greater as compared to the other breeds. This suggests that Toda is a different breed, which may warrant its germplasm conservation and management as a separate unit[9]. However, it would be equally important to substantiate the observation employing other approaches that Toda is a separate unit. In this context, simultaneous analysis of several VNTR loci[61], mt genome[62], sequences coding for cytochrome b may be undertaken for analysis[51]. Similarly, analysis of polar and non-polar mutation, divergence of consensus and core element and their comparison amongst different breeds of buffalo would assist delineating different breeds[2].

It may be noted that genetic changes that percolate at the breed level may not be easily identifiable due to technical limitation of one approach. Thus, a multiplex approach is envisaged to augment the breed delineation. In the context of molecular mining, a brief account of PCR based minisatellite associated sequence amplification (MASA)[51] is presented.

PCR mediated genome analysis

The sensitivity, speed and versatility of the polymerase chain reaction (PCR) have facilitated the experimental approaches to genome analysis, forensic sciences and evolutionary biology. Unlike conventional PCR, Arbitrarily Primed Polymerase Chain Reaction (AP-PCR), also referred to as Random Amplification of Polymorphic DNA (RAPD)[63], performed with a single primer, generates discernible amplicons. The RAPD reactions are dependent on the Mg++ concentration, length and sequence complexity of the oligo primer(s) and their annealing temperatures with target DNA[64]. Unlike restriction fragment length polymorphism (RELP) which requires at least 100 folds intact DNA, and RAPD reaction can be performed with much smaller quantity of target substrate even without prior information on the organization of genome. The primer sequences used in RAPD reaction, not shared by many species, have their applications confined to a single or limited number of genomes. On the other hand, primers based on evolutionarily conserved or "largely shared" sequences are envisaged to be useful for analyzing a large number of genome[65,66]. Analogous to RAPD or AP-PCR amplification, we used well-defined primers originating from the minisatellite regions of human[53] and animal genomes[67] for minisatellite

associated sequence amplification (MASA) with genomic DNA from a number of species. MASA generated amplicons by evolutionarily conserved primer(s) or sequences shared by many species are particularly useful for clad identification in controversial systematics, comparative genome analysis, and for establishing the phylogenetic status of an organism in addition to wildlife conservation biology and forensic medicine.

Reaction conditions for minisatellite associated sequance amplification (MASA)

MASA reactions were carried out in a 25 µl volume containing 25 ng of genomic DNA as template, 20 pmoles of primer, 2.5 units of Ampli*Taq* DNA polymerase (Perkin Elmer Cetus, USA), 2.5 mM $MgCl_2$, 200 µM of each dNTP, 0 mM KCl, 20 mM Tris-HCl (pH 8.3) and 0.1% Triton X-100. The reaction mixture was layered with an equal volume of mineral oil and heat denatured at 96°C for 2 min. Following this, amplification was carried out for 35 cycles comprising subsequent steps of denaturation at 94°C for 1 min, annealing at the optimal temperatures for 1 min and extension at 72°C for 1 min in a Thermal Cycler (Perkin Elmer, Cetus). On completion of the cycles, the amplified products were further incubated at 72°C for 5 min. The resultant amplicons resolved on the agarose gel uncovered species-specific band profile (Fig. 4)

Minisatellite sequences have been implicated with high rate of recombinatorial activities leading to sequence polymorphism in the genome[68]. Thus, employing MASA approach with primers originating from different types of repetitive DNA, bubaline genome may be studied for establishing the individual hypervariable repeat loci. This in turn will provide an opportunity to assess if any of the loci may be interpreted in the context of QTL loci or other physical or physiological attributes. Clearly, PCR technology may have numerous applications leading to different aspects of bubaline genome analysis but we have confined our discussion within the realm of repetitive DNA.

Owing to genome specific amplicons generated by MASA with defined primer(s), we envisage its potential use in the comparative genome analysis and clad identifcation. However, it would be unattainable to acurately position a clad based on the amplicons generated by a single repeat motif. Thus, for more accurate clad positioning or phylogenetic analyses, several coding and non- coding loci may simultaneously be analyzed, MASA mediated genome specific amplicons by primer(s) shared amongst a large number of species adds a new dimension to molecular systematics of advanced eukaryotes.

Analysis of the bubaline genome in the present day context and possible areas of research

Bubaline, despite being an important species has not attracted much attention for molecular genetic studies. Perhaps, unlike Drosophila and mouse, this animal wasnot found feasible as experimental model owing to logistics of its upkeep and propagation at par with

other smaller animals. However, this seems to be intriguing because a number of diary farms in the country do provide an opportunity to undertake detailed study on this animal. With the fast changing global economic scenaries and ever growing menace of patenting superior genetical biodiversity by the so-called advanced countries, it has now become absolutely crucial to re-assess our research priorities retrospectively in the areas of genetics of farm animals and animal biotechnology. What needs to be done immediately is to create a far higher level of awareness amongst committed scientists to make them realize the relevance of research not only on bubaline but also on other important farm animals. The single guiding source of research on this species should be that information so generated must ultimately culminate in assisting the fruitful manipulation of the genome. Clearly, genome manipulation at any level for any purpose (e.g. generation of transgenic animals with desired physical and physiological attributes) will be feasible only if a great deal of base line date is available. Obviously, prioritization of research on buffalo is not the priority of this article and that must be left to the workers. Ideally, understanding all the expressed genes and their mode of actions and interactions within the bubaline genome will positively bridge the gap and facilitate the much-needed advanced research. Alternatively, characterization of genes in order of priority will prove to be equally useful. This may include genes controlling phenomenon of sex determination, spermatogenesis growth, lactation and fecundity. Equally important will be the studies on MHC locus to uncover the organization and expression of genes and their exon with spacer or repetitive sequences. Genetic factors that are known to be involved in conferring resistance to diseases for e.g. in other living systems may be studied in bubaline genome.

Concluding Remarks

1. Despite its importance, research data on the bubaline genome is negligible which would be possible if higher level of awareness is brought amongt workers.

2. Detailed information on different breeds and their genetic basis must be established so that the same may be exploited to maximum.

3. All the structural and regulatory genes tagged with satellite sequences may be studied in detail so that assessment of segregation of such genes in actual farm animals at the genetic level becomes feasible.

4. Loss of hybrid vigor and increased level of homozygosity may be assessed at the DNA level to establish a correlation with the physical and physiological attributes.

5. Finally, emergence of any relevant data on the molecular genetics of bubaline genome will automatically strengthen the much needed gap in the area of animal biotechnology in general and analysis of bubaline genome in particular.

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Surface ultrastructure of the male external genitalia in the rock-honeybee, *Apis dorsata* F. (Hymenoptera : Apidae)

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Abstract

In the rock honeybee, *Apis dorsata* the male external geitalia consists of an aedeagus internally and a phallotreme along with a pair of penis valves and gonoconites on the 9th sternum externally. The aedeagus is well differentiated into an apical penis bulb, a middle cervix and a distal vestibulum. The penis bulb consists of one mid dorsal, and two lateral cuticular plates. The cervix bears s fimbricated lobe. The wall of the cervix is externally covered with the bristles and scales while inner surface is lined with the sensory pegs, papillae, pits with bristles and microtrichia. The vestibulum bears bristles all over the external surface except the middle membranous wrinkled region. The cornual appendages are externally covered with the fine triangular scales. The phallotreme is guarded with the pair of penis valves which are externally covered with the fine bristles, scales and globular microtrichia on their inner margin. The gonocoxites are covered externaly with the bundles of long hairs. Functional and species-specific significance of the fine cuticular processes has been discussed.

(**Keywords:** *Apis dorsata*/ male external genitalia/ aedeagus)

Introduction

Snodgrass[1] has described thoroughly the morphological structure of the male external genitalia in *Apis mellifera.* Later on, some workers[2-7] have given an account on that of the other species of honey bees including *Apis dorsata* but very meager information is still available on the fine cuticular processes pertaining to the surface of various external genital organs. The present studies were, therefore, undertaken to explore surface ultrastructure of the male external genital organs of *Apis dorsata* in order to understand their fine structure, functions and taxonomic significance.

Materials and Methods

A large number of adult drone bees (*Apis dorsata*) were collected from the old colonies during 1991-1993 from the forest region of the Central India. The external genitalia was dissected out, the penis bulb, fimbricated lobe and cornuae were separated, fixed in the

aqueous Bouin's fluid for a period of 18-24 hrs, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax (62°C). The 4μm thick sections were cut and stained with the Heidenhain's Iron-Haematoxylin – Orange-G staining technique[8].

For surface ultrastructure of various organs of the male genitalia, the dissected genital organs were washed in distilled water, boiled in aqueous 10% KOH solution till the material became fully transparent. The mateirtal was thoroughly washed, dehydrated, cleared in acetone and mounted on the stubs at different angles with an adhesive, Fevicol. The mounted material was coated with gold in the 'Paloron-gold coating unit' and scanned under the Sterioscan 250 MK III cambridge Scanning Electron Microscope at desirable magnifications[9] at The Regional Sophisticated Instrumentation Centre, Nagpur University Campus, Nagpur (India).

Results

The external male genitalia consists of a large, highly evolved and complex aedeagus and slit-like orifice, the phallotreme terminally. The phallotreme is guarded by a pair of penis valves laterally and a pair of gonocoxites mid-dorsally (Fig. 1).

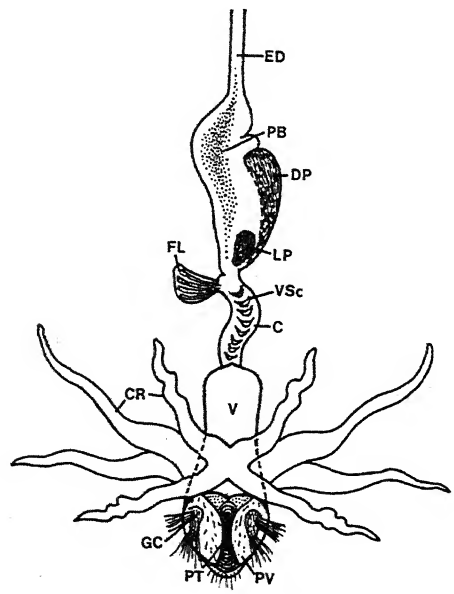

Fig. 1 – Anatomical organization of the male external genitalia in *Apis dorsata*. Co-cervix, CR-cornuae, DP-dorsal plate, ED-ejacuatory duct, FL-fimbricated lobe, GC-gonocoxite, LP-lateral plate, PB- penis, PT-phallotreme, PV-penis valve, V-vestibulum, VSc-ventral sclerites.

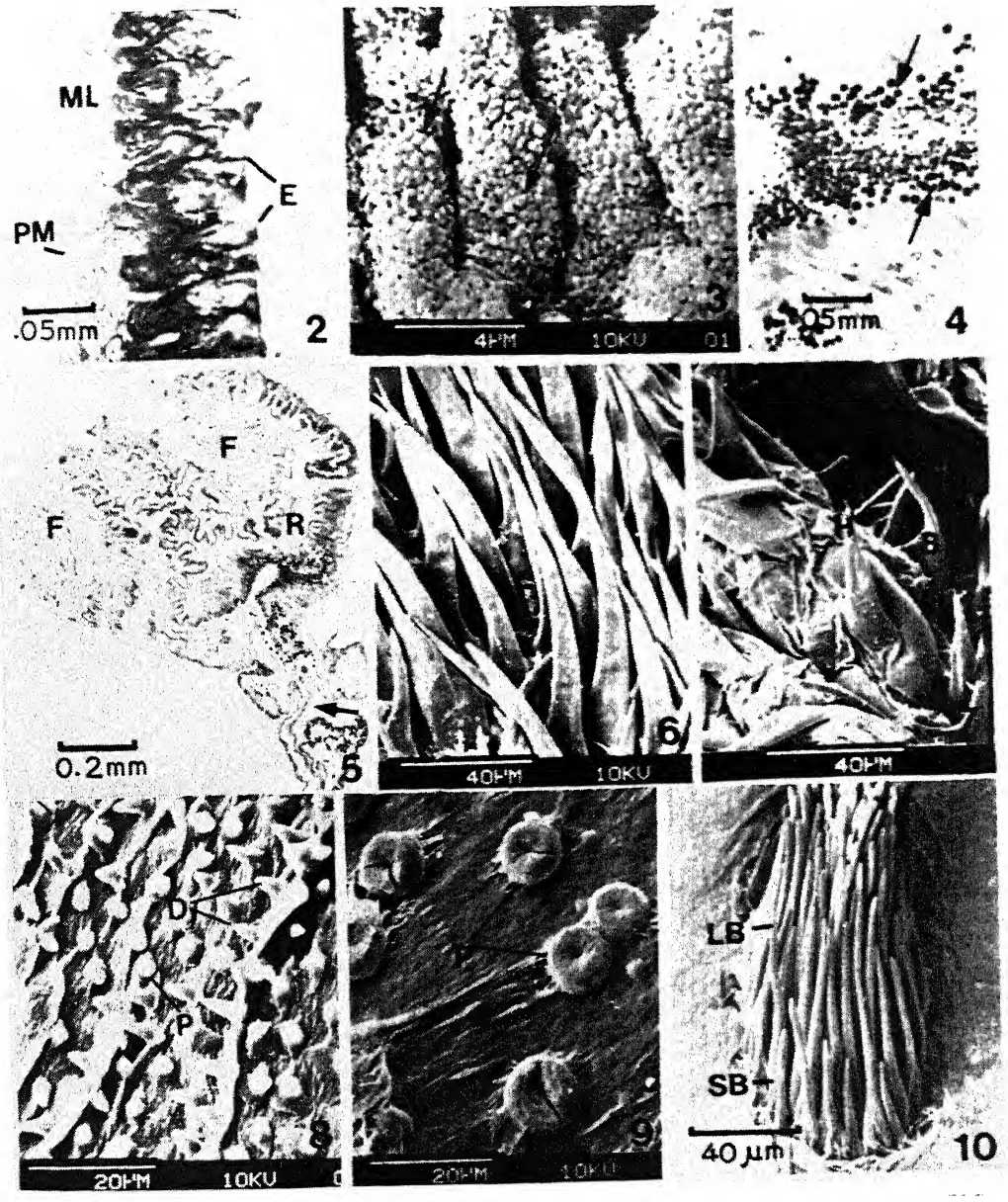

Fig. 2 – Longitudinal section of penis bulb showing the wall composed of an outer peritoneal membrane (PM), middle muscle layer (ML) and inner epithelium (E).

Fig. 3 – SEM of inner surface of a wall of the penis bulb showing longitudinal rows of the beaded structures.

Fig. 4 – Longitudinal section of the posterior region of penis bulb showing darkly stained secretory droplets in the lumen (arrows).

Fig. 5 – Longitudinal section of the fimbricated lobe bipectinate fimbrea (F) on either side of raches (R) and the stalk of a fimbricated lobe with a central duct opening into the lumen of the cervix (arrow).

Fig. 6 – SEM of dorsal surface of cervix showing flattened bristles.

Fig. 7 – SEM of of postero-ventral region of the cervix showing bristles with broad base (B) and short hairy process (H).

Fig. 8 – SEM of internal surface of anterior region of cervix showing rows of denticles (D) and papillae (P).

Fig. 9 – SEM of internal surface of middle region of cervix showing the papillae only.

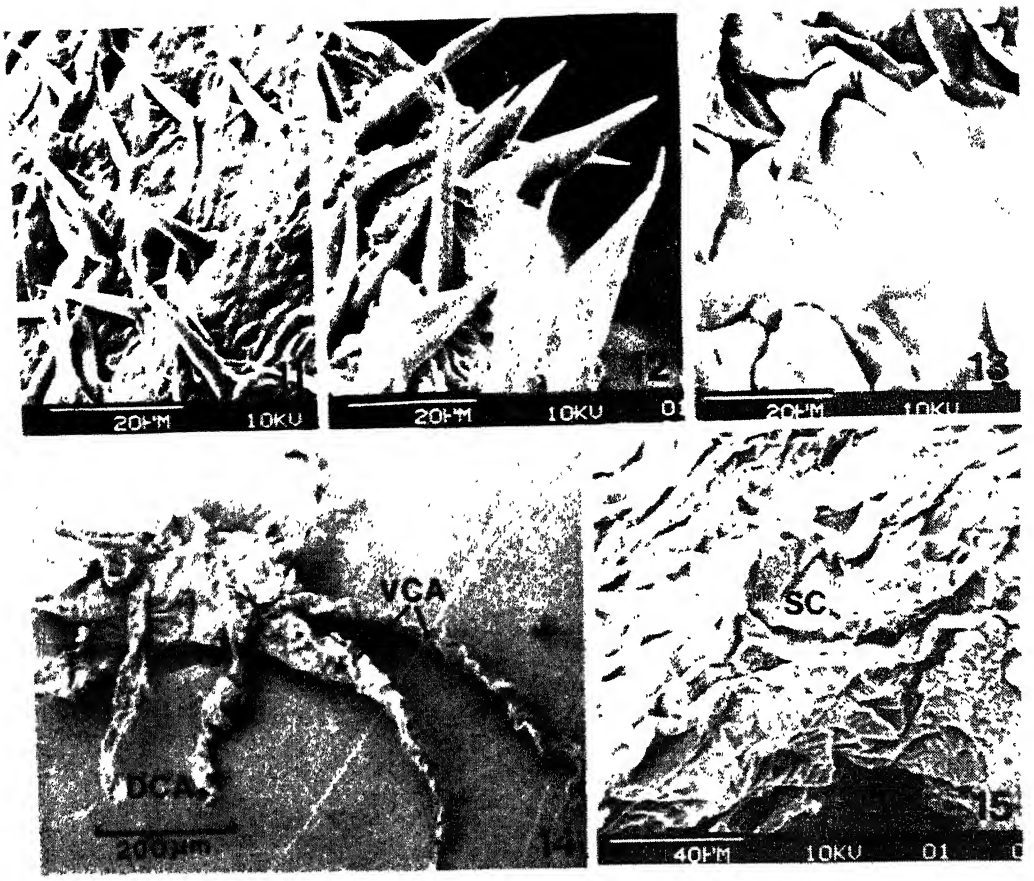

Fig. 11 – SEM of the internal surface of ventral wall of cervix showing microtrichia.
Fig. 12 – SEM of the external surface of vestibulum showing broad bristles.
Fig. 13 – SEM of the mid-ventral region showing membranous wrinkled surface.
Fig. 14 – SEM of the cornuae consisting dorsal (DCA) and ventral (VCA) appendages.
Fig. 15 – SEM of cornuae showing wrinkled external surface and scales (SC).

The aedeagus lies in the ventral abdominal region as the endophallus while during copulation it everts outside the body through a phallotreme and becomes the ectophallus, *i.e.* the active penis. The aedeagus is about 6.0 ± 0.5 mm long and extended from the 2nd to the 9th abdominal segments. It is differentiated into three regions–an apical penis–bulb, a middle cervix and a distal vestibulum.

The penis-bulb (PB) is an oblong sac-like structure. The wall of the penis-bulb is composed of an outer peritoneal sheath, a middle muscle layer and an inner epithelial layer. The epithelial layer consists of the tall epithelial cells and it is internally lined with a thin cuticular initima (Fig. 2). It gives out a dorsal and apair of lateral plates at the opening of the PB into the cervix. The wall of PB is folded internally at some places. The cuticular intima is differentiated into fine spherical structures giving beaded appearance to the entire surface (Fig. 3). The lumen of the PB is filled with intensely stained droplets of the secretory material in the distal region (Fig. 4). In the post-copulated drones, the lumen is filled with sperm bundles along with mucus gland secretory material.

The fimbricated lobe (FL) is situated dorsally at the junction of the PB and cervix. It is a hollow globular organ with a short basal stalk. Interally, it consists of 4 longitudinal raches, each bearing large number of bipectinate fringes, the fimbrea (Fig. 5). The outer raches are longer than the inner ones and open into a common central duct of the stalk running to the lumen of the cervix.

The cervix is a long and partially twisted tubular structure. The outer surface of the cervix is covered dorsally with the long bristles of about 76 ± 6.67 µm length (Fig. 6) and the 'V'-shaped scales are situated mid-ventrally. The apical and distal ventral regions of the cervix are covered with the bristles with flattened base and short hairy process (Fig. 7). The inner surface of the wall of the cervix is lined with the sensory pegs and spherical papillae of about 6.22 ± 0.69 µm in length and 4.47 ± 0.45 µm in diameter, respectively (Figs. 8, 9). The mid-ventral wall of the cervix contains internally the pits bearing a large number of short and long bristles of about 37.33 ± 3.50 and 65.50 ± 4.75 µm in length, respectively (Fig. 10). These bristles are originated from the inner surface of the 'V'-shaped scales. The rest of the inner surface of mid-ventral and that of the dorsal wall of the cervix possesses a large number of the microtrichia of about 15.52 ± 1.72µm in length (Fig. 11).

The vestibulum is a short pouch-like structure and represents the posterior-most part of the aedeagus. It is exernally covered with the basally flattened membranous bristles (Fig. 12) except the mid-ventral region, which is covered with the membranous folds (Fig. 13). The vestibulum bears two pairs of cornuae mid- dorsally, one on dorsal and another on the ventral side. Each cornua consists of a bilobed flattened base and two pairs of apical tubular appendages (Fig. 14). The appendages of the dorsal cornuae are short while that of ventral

prime function of sperm transfer. The presence of the globular microtrichia on the margins of the PV of *A. dorsata* has been described, perhaps, for the first time.

Acknowledgment

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Sperm abnormality induction assay of the drug diclofenac sodium

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Abstract

The non-steroidal anti-inflammatory drug Diclofenac sodium was evaluated for its possible in *vivo* mutagenic effect through sperm anomaly assay in albino mice *Mus musculus*. Three different doses of the drug viz., 2.5, 5.0 and 10.0 mg/kg body weight were administered through intraperitonial injection. Male mice were sacrificed after 35 days of injection. Adequate number of distilled water treated (places) and an anticancer drug cyclophosphamide (50 mg/kg) administered positive control mice were sacrificed similarly. Sperm suspension was obtained from cauda epididymis. It was stained with aqueous eosion and was smeared on cleanslides. Body weight, testes weight and sperm count were calculated. Different types of abnormal sperms viz., hookless, amorphous, folded, banana, double tailed/headed etc. were scored. The data were subjected to Mann- Whitney-U-test. There was no significant difference in the body weight, testes weight and sperm count. But the significant incidence of abnormal sperms which was dose dependent showed Diclofenac sodium to be mutagenic in the present test system.

(**Keywords:** sperm anomaly/mutagenic/diclofenac sodium).

Introduction

Diclofenac sodium (CAS No. 15307–79–6, Dominion Chemical Industries Limited, Batch No. D5666) is a nonsteriodal antinflammatory drug (NSAID) used in the treatment of rheumatic arthritis and other diseases. It is sold in the trade name Voveran. It is the eighth largest selling drug and the most frequently used NSAID in the world[1]. It is chemically sodium [2-(2, 6- dichloroanilinophenyl)] acetate and is a phenylacetic acid derivative with the molecular formula $C_{14}H_{10}Cl_2NNaO_2$ and molecular weight 318.13. Diclofenac sodium blocks the cyclooxygenase pathway leading to the synthesis of prostoglandin and thromboxane responsible for several inflammatory effects. It also inhibits the lipooxygenase pathway thereby inhibiting the formation of LTB , a known pain mediator.

It is known that many substances with an anti-inflamatory action influence DNA metabolism and can thus give rise to damage in the genetic material[2]. Due to this fact, analysis of interaction of diclofenac sodium with genetic material is very relevant. Since

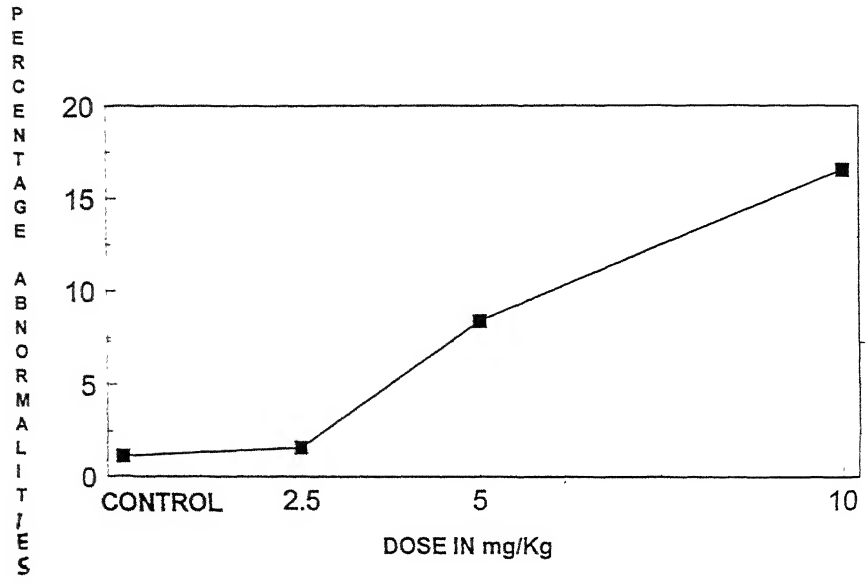

Fig. 1 – Dose-effect curve of total sperm abnormality by diclofenac sodium along with control values.

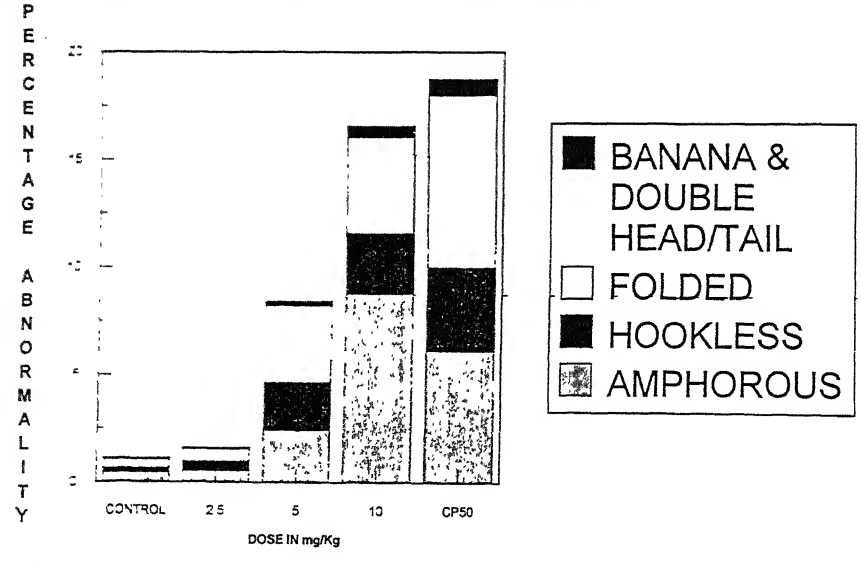

Fig. 2 – Illustrating the distribution of different types of abnormal sperms in diclofenac sodium treated & control animals.

there is no detailed report on the direct *in vivo* mutagenic potential of it in mammalian system, an attempt is made to study the possible genotoxic effects of this drug in mouse *Mus musculus* using *in vivo* sperm morphology assay.

Materials and Methods

Swiss albino mice of the *Mus musculus* species of 9-10 weeks old with an average body weight of 26 ± 2 gm that were inbred, maintained and fed with the standard mouse pellets (Lipton India Ltd.) and water *ad libitum* were used. Five male mice were used for each treatment and control group. Three different doses of diclofenac sodium viz., 2.5, 5.0 and 10.0 mg/kg body weight were administered as intraperitoneal injections in 0.2 ml quantity diluted with distilled water (sterile) to animals of individual dosage groups. The lowest dose of 2.5 mg/kg body weight corresponds to the therapeutic dose of human. Animals were sacrificed after 35 days (5 weeks) alongwith distilled water treated negative control groups. Similarly, animals injected with 50 mg/kg body weight of cyclophosphamide [(CAS No. 6055-19-2) Endoxan Asta-Medica AG, Germany, a known mutagen were sacrificed to act as positive control.

It takes 35 days for the conversion of sperm mother cell to sperm in mice. Hence the protocol is useful to assess the effect of drug on the development of sperms. Initial body weight of the test animals before the administration of drug and the final body weight before sacrificing were noted. The cauda epididymis were separated and the sperm suspension was prepared. The filtered suspension was stained with aqueous eosin Y of 1:10 dilution with distilled water. A uniform, thin smear of stained suspension was made on clean slide. 2000 sperms per animal were analysed for the abnormal shapes following the criteria described by Wyrobek and Bruce[3]. Testis weight and sperm count per epididymis were also determined following the method of Vega *et al.*[4]. The statistical significance of the data were calculated using Mann- Whitney U-test[5].

Results

The sperm morphology assay revealed the incidence of different types of abnormal sperms like banana, hookless, amorphous, folded, double head/tailed forms in different doses of Diclofenac sodium treated mice (Fig. 1). The percentage frequency of abnormal sperms induced are represented in the Table 1. Diclofenac sodium induced significant frequency of sperm shape abnormality in all the doses. There was a dose dependent increase in the total aberration percentage as shown in the graph. Amorphous, and folded types of sperms were predominant in all the doses tested, and the banana shaped sperms at the lowest percentage (Fig. 2). The individual type of anomalies were statistically significant in both 5 mg and 10 mg/kg dose treatment. 50 mg/kg endoxan treated positive control mice showed statistically significant frequency

Table 1 – Percentage frequency of different types of abnormal sperms induced 35 days after treatment with Diclofenac sodium and controls[++]

Dose in mg/kg	Amarphaus ± SEM	Hookless ± SEM	Folded ± SEM	Banana ± SEM	Double Head ± SEM	Total ± SEM
Control	0.45 ± 0.1	0.18 ± 0.11	0.43 ± 0.09	0.03 ± 0.06	0.04 ± 0.02	1.13 ± 0.05*
2.5	.054 ± 0.12	0.42 ± 0.07	0.59 ± 0.02	0.02 ± 0.08	0.04 ± 0.09	1.61 ± 0.22*
5.0	2.42* ± 0.08*	2.2 ± 0.03	3.6 ± 0.16	0.03 ± 0.12	0.17 ± 0.01	8.42 ± 0.28*
10.0	8.79 ± 0.13*	2.76 ± 0.18*	4.49 ± 0.11*	0.14 ± 0.06*	0.36 ± 0.04*	16.54 ± 0.17*
Endoxan 50.0	6.11 ± 0.09*	3.88 ± 0.15*	8.0 ± 0.2*	0.24 ± 0.14*	0.5 ± 0.21*	18.73 ± 0.91*

Table 2 – The effect of different doses of diclofenac sodium on mean body weight, difference in testis weight and epididymal sperm count, 5 weeks after treatment alongwith control values.

Dose (mg/kg)	No. of animals	Initial body wt. in gm ± SEM	Final body wt. in gm ± SEM	Difference in wt. in gm ± SEM	Testis wt. in gm ± SEM	Sperm count /Epididymis	% Abnormal sperms ± SEM
Control	5	23.8 ± 0.36	26.8 ± 0.80	3.0 ± 0.40	0.21 ± 0.09	3170000	1.13 ± 0.27
2.5	5	27.4 ± 0.34	30.6 ± 0.86	3.2 ± 0.27	0.21 ± 0.03	8170000	1.61 ± 0.20
5.0	5	26.8 ± 0.30	29.7 ± 0.40	2.9 ± 0.70	0.21 ± 0.07	8500000	8.42 ± 0.76
10.0	5	26.2 ± 0.22	28.1 ± 0.52	3.9 ± 0.90	0.20 ± 0.01	7490000	16.54 ± 0.68
Endoxan 50.0	5	28.0 ± 0.68	33.4 ± 0.47	5.4 ± 0.34	0.20 ± 0.06	1820000	18.73 ± 0.90

of total and individual type of abnormal sperm morphologies compared to vehicle treated control which was also higher than diclofenac sodium treated mice. The data pertaining to the effect of diclofenac sodium on the body weight, testis weight, and sperm count per epididymis are given in Table 2. There was dose dependent decrease in the testes weight, and sperm count although not significant statistically. There was no significant difference in the body weight.

Discussion

Sperms are important indicators in reproductive toxicology as they can be used to assess the spermatogenic damage, the fertility effects and the heritable mutations induced either by physical or chemical mutagens. In the present study, the frequency of abnormal sperms were statistically significant at 5 weeks after treatment of different doses of Diclofenac sodium including the therapeuic dose. It indicates the damage induced by the drug at the spermatocyte stage. The frequency of sperm shape abnormality showed a dose dependent increase and the total sperm abnormality percentage at 10 mg/kg was 16.54. This observation indicates the accessibility of the compound and/or its metabolite viz. hydrox and dihydroxy diclofenac to the testes.

According to Wyrobek *et al.*[6], several kinds of mutation can lead to abnormal sperm morphology. Hence, this test is considered more sensitive in detecting germ cell mutagens than other germinal mutagenicity assay. The abnormalities in sperm is said to arise due to specific point mutation, probably small deletion or both[7]. Y chromosomes in addition were reported to have an important role in determining the total percentage of sperm head abnormalities[8]. Brinkworth *et al.*[9], were of the opinion that protein abnormality may indicate abnormal sperm shape as sperm shape is partially imparted by structural proteins.

The testis weight and sperm count served as the additional parameters of sperm production. Both the testis weight and sperm count decreased with the increased drug dose. The loss of testis weight is, as a result of a loss of the germ cells[10]. The reduction in sperm number is associated with reduced fertility. Although sperm count is not as sensitive to small changes as sperm morphology, it is a responsible indicator of male reproductive toxicity[6]. It may be concluded that Diclofenac sodium is a germ cell mutagen in mouse *in vivo* system.

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Re-examination of the microvasculature of the pseudobranch of *Mastacembelus armatus (Lac.)* and *Channa punctatus*

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Abstract

The pseudobranch of *Mastacembelus armatus* (Lac.) and *Channa punctatus* were reexamined for detailed study of their microvasculature in order to throw light on relationship between the pseudobranch and the cartoid labyrinth. The findings show that in case of *C. punctatus*, the pseudobranch combines the shunt vessels of cartoid labyrinth with the sheet flow microvasculature of the pseudobranch. The results are discussed in light of homology between the two.

(**Keywords :** pseudobranch/carotid labyrinth/shunt vessels/ *Channa punctatus/Mastacembelus armatus*)

Introduction

Pseudobranch present in most teleosts may occur in two forms; free or covered (buried). The histological organization, though basically hemibranch–like in the former is modified in the latter and varies from species to species. A constant feature of pseudobranch, however, is the presence of pillar cells even where the pseudobranch is modified beyond recognition and has become glandular. However, the most characteristic cells in its histology are the pseudobranchial cells that line the basal lamina opposite the pillar cell system of microcirculation.

Pseudobranch is known to be highly plastic.[1,2] Interest in the study of pseudobranch has been recently aroused for 3 reasons : (i) Pseudobranch of some teleosts reflect a ''sub-carotid labyrinth'' stage of organization with regard to the hypothesis of pseudobranch transformation into carotid labyrinth; (ii) Pseudobranch and cartoid labyrinth are homologous, being both derived from the posterior hyoidean hemibranch[3,4]; (iii) structural variation presents ambiguity with regard to evaluation of function of the pseudobranch.[5] There is a need to examine new species as well as to reexamine species in which case information is inadequate. Work of *Mastacembelus armatus* remains incomplete, as only its early development has been investigated.[6] Work on *Channa* sp.[7] including corrosion

cast replica examination[5] also calls for reinvestigation. The present work reports the findings on the adult pseudobranch of *Mastacembelus armatus* and *Channa punctatus*.

Material and Methods

Fishes used in the investigation were captured from the local rivers. They were anesthetized with 0.02% tricaine methanosulphate (MS. 222 Sandoz) or benzacaine and dissected under zoom binocular. The palate was dissected and the swelling of the pseudobranch was located. The entire structure was removed along with the bases of the connecting arteries associated with it. The excised material was fixed in Bouin's fluid by immersion and processed for paraffin microtomy in the routine way. Serial sections 6-8 μm thick were cut and stained in Azan-Mallory's triple stain and Haematoxyline Eosine stain.

Results

Pseudobranch of *Mastacembelus armatus* (Figs. 1-2)

The only account of the presence of pseudobranch in this species is available in the work of Bhargava,[6] which is, however, a very short description giving no details of the microvascular architecture. In view of the limited scope of the present work it is not intended to describe the details of the organization of the pseudobranch but to put forth only the organization of microvasculature. Thus, it would suffice to say that the general layout and organization of the pseudobranch of this species (Fig. 1) resembles those of most of the other teleosts, e.g. that of *Anabas testudineus*[8,9]. The "hair pin loops" of the secondary lamellae make a densely compact arrangement. Afferent supply and efferent collection of blood is carried out by arteries situated in the medula and cortex respectively. The microvasculature consists of pillar cell system of sheet flow in the secondary lamellae carrying well developed pseudobranchial cells on either side. Presence of pillar cells is clearly discernible (Fig. 2).

Pseudobranch of *Channa punctatus* (Figs. 3-8)

For account of macrovascular architecture as well as microvascular architecture of the pseudobranch of this species the reader is referred to the earlier work of Tripathi[9] and Srivastava *et al.*[7] The present observation is confined to verification of the description of micro-vasculature reported earlier, on one hand, and to bring to light any other feature of micro- vasculature which has not been reported earlier, on the other hand. The present observation on the general layout of the secondary lamellae (Fig. 3) is in agreement with the previous description. However, an interesting additional feature revealed is the arrangement of "hair pin loops" in opposite rows (Fig. 4). The most outstanding feature,

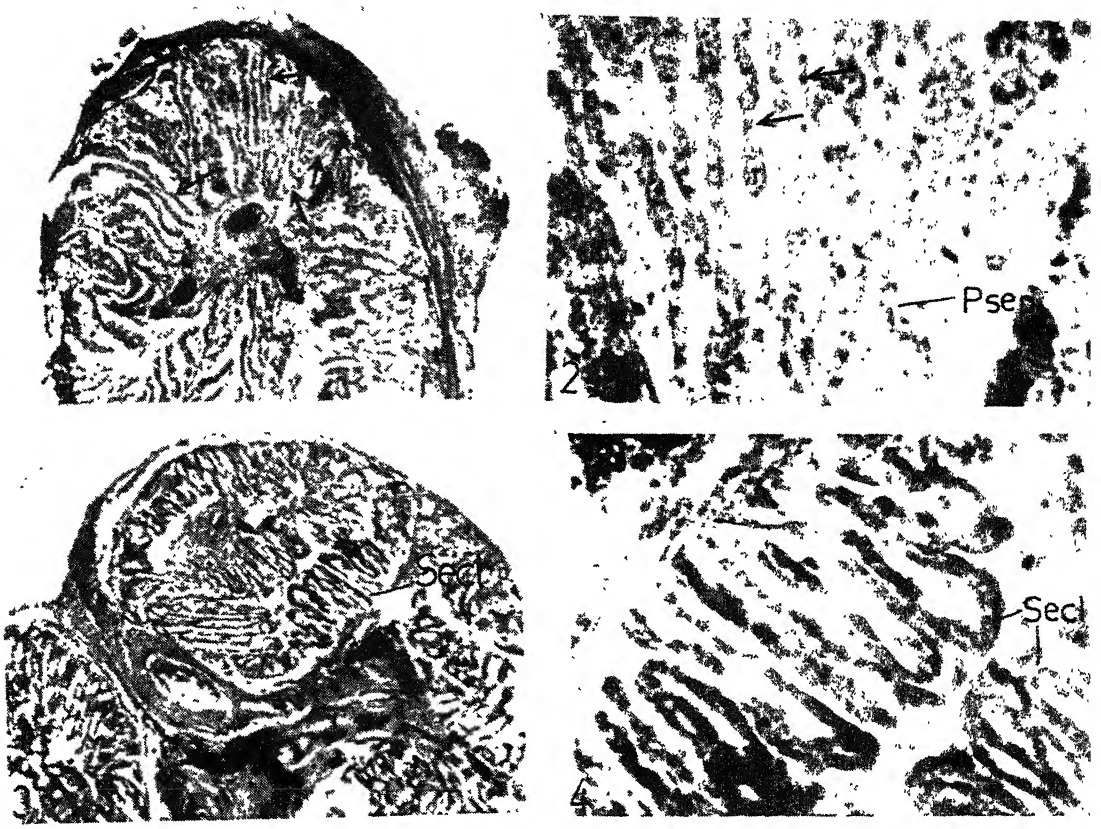

Fig. 1 – Cross section of the pseudobranch of *Mastacembelus armatus* showing the normal distribution of secondary lamellae (simple arrow) in a lobe Mallory's triple stain, x 300.

Fig. 2 – Cross section of the pseudobranch of *Mastacembelus armatus* showing the "hair pin loops" of secondary lamellae and pseudobranchial epithelial.
Note : The presence of pillar cells (simple arrow) in the capillary-like vessels. Psep. Pseudobranchial epithelium Triple Mallory's stain x 750.

FIg. 3&4 – Cross sections of the pseudobranch of *Channa punctatus*, showing normal disposition of the secondary lamellae in the lobes.
Note : Opposite rows of "hair pin loops" of the secondary lamellae Secl, secondary lamellae, Fig. 3 Haematoxylin eosin stain X 400 and Fig. 4 Haemotoxylin eosin stain X 400.

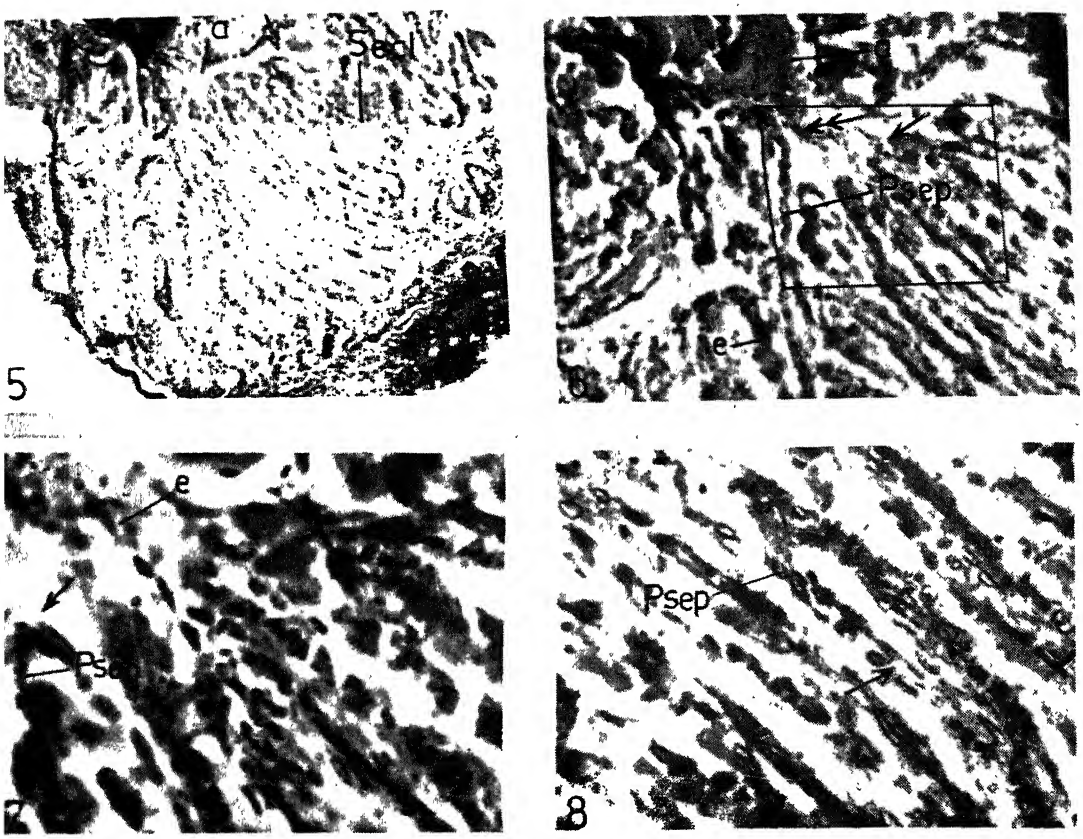

Fig. 5 – Cross section of the pseudobranch of *Channa punctatus* showing branching capillaries commmunicating with "hair pin loops" of the secondary lamellae a, artery, secl, secondary lamellae, Haematoxylin eosin stain X 400.

Fig. 6 – Cross section of the pseudobranch of *Channa punctatus* showing the connection between a group of "hair pin loops" with artery through an arteriole (double headed arrow) and its branching capillaries (arrow) a, artery, Psep, pseudobranchial epithelium e, endothelium. Haematoxylin eosin stain X 750.

Fig. 7 – Cross section of thepseudobranch of *Channa punctatus* showing magnified view of a portion of Fig. 6 mark with rectangle. e, endothelium, Psep, pseudobranchial epithelial Haematoxylin eosin stain X 900.

Fig. 8 – Cross section of the pseudobranch of *Channa punctatus* showing adjacent secondary lamellae. The absence of pillar cells in the capillary like profiles (arrow) Psep, pseudobranchial epithelium, Haematoxylin eosin stain X 900.

not known hitherto, is the revelation that a system of branching capillaries supplies blood to each set of "hair pin loops" of secondary lamellae (Fig. 5). The capillary system branches out from a short arteriole branch which in turn branches out (Fig. 6) from a main artery. The capillary lumen is lined by endothelial cells (Figs. 7-8). Another interesting feature is the verification of the earlier observation of total absence of pillar cells in the sheet flow system of secondary lamellae, as revealed by very careful and close examination.

Discussion

The covered pseudobranch of *Mastacemelus armatus* is typically organized with a system of pillar cells sheet-flow channels, similar to that of the majority of teleosts.[10,11] Secondary lamellae are folded in a hair-pin loop fashion, displaying pilaster cells and pseudobranchial epithelial layers but no interlamellar space similar to the condition found in *Anabas testudineus.*[7]

The covered pseudobranch of *Channa punctatus*, however, reveals very interesting features, not reported hitherto.

1. The secondary lamellae are thrown into "hair-pin loop" arrangement as reported earlier.[7,5] The view of 2 directional counter current multiplier system of microcirculation for the pseudobranch[5] is supported.

2. Pilaster cells are found lacking in conformity with the previous observation of Srivastava *et. al.*[7] but in total disagreement with Roy *et. al*[5] There is, therefore, a need for verification of the presence or absence of pilaster cells by electron microscopical observation before the so-called "sub- carotid labyrinth" status[7] may be accepted.

3. The presence of branching endothelium, lining terminal arterioles and capillaries feeding the hair-pin loops foldings of the secondary lamellae, is very significant. This feature indicates the suppression of pillar cell system and its replacement by endothelium-lined microvessels. It is likely that the teleostean pseudobranch evolved several times independently of each other.[10,12] Whereas in majority of cases of covered pseudobranch viz. *Anabas* sp. pillar cell sheet flow system survived, in the case of carotid labyrinth the pillar cell system was completely replaced by the arterio-arterial microcirculation. In *Channa punctatus,* as an intermediate stage, both are retained as the replacement was partial—a step in the direction of evolution of carotid labyrinth. It may be noted that when carotid labyrinth is present, viz. catfish, pseudobranch is absent. Even in a basic gill organisation, whereas the sheet-flow pillar cell supported channels are present in

the exposed part, arterioles do form a plexus in the unexposed part.[13] When gill lamellae are suppressed for one reason or the other, arterioles become important and replace the pillar cell sheet flow by capillary plexus, viz. shunt vessels in small buds on 2nd gill of *Monopterus cuchia*[11], islets on 3rd and 4th gills of *Anabas testudineus*[14], buds of atrophied 4th gill of *Channa striatus*[15] and arborescent gill of dipnoans.[12] In fact, the presence of endothelium-lined marginal channels[13] continuous with the sheet flow pillar cell supported channels in the gill itself is reminiscent of partial replacement of the former by the latter or partial restoration of the latter. Even during development, the endothelium lined marginal channel appears before the development of pillar cell system.[16] The current hypothesis on the origin of pillar cells holds that the pillar cell system of sheet flow is derived from a system of arterial endothelial-lined capillaries in which endothelium was lost.[17] Thus the marginal channel could be the representative of a more primitive condition from which source elaborated the arterio- arterial plexus of shunt vessels : arterioles, terminal arterioles, metaorterioles and capillaries of the carotid labyrinth[4] on the one hand, and the pillar cell sheet flow system of the pseudobranch on the other hand. The microvasculature of the pseudobranch of *Channa punctatus* combines both the features.

The findings favour the view of Srivastava (1994) that pseudobranch and carotid labyrinth are homologous, being derived from gill. Of the three alternative gill precursors proposed for the carotid labyrinth microvasculature,[4] namely the shunt vessels, the marginal channel and the cavernous body, the present finding lends support in favour of the shunt vesseles.[18,19]

Acknowledgements

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Bacterial population and diversity on "Fiddler Crab" (Uca sp.) at Bhavnagar coast

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Abstract

Bacteria colonizing the body parts, viz. gill, carapace, abdomen and gut of "fiddler crab" (*Uca* sp.) were examined for their composition and distribution during various seasons for 2 years. Vibrios, micrococci and pseudomonads were the most dominant organisms which attained maximum population size during and after monsoon months. In frequency index the 17 bacterial species representing 10 genera varied between common to occasional onthe different body parts. The Shanon Index indicated carapace to be the most diverse habitat.

(**Keywords:** bacterial population/fiddler crab/crustacean)

Introduction

The marine animals practically bathe in suspension of microorganisms, especially bacteria and fungi. But how far they interact with them and with what consequences, is difficult to assess. Their association may be of a variety of types–some microorganisms just pass by without any interaction, while others colonize the surface or accumulate at the site of any bruise or damage under the influence of some leaking chemicals, and become resident microbiota. They can also enter the body through the mouth and elicit varied responses. Knowledge on the microbiology of animals in marine ecosystem is very deficient as compared to informations available with terrestrial ones, and the informations that are available are only for fishes[1-3].

In this study we have examined the bacteria associated with a crustacean,the "fiddler crab" (*Uca* sp.), which is most common at Bhavnagar coast and is eaten like a fish.

Materials and Methods

Fiddler crabs were collected at bimonthly intervals from November 1996-November 98 near Ghogha port, Bhavnagar with help of fisherman, in sterile polythene bags and brought to the laboratory in ice-boxes. The various parts (carapace, gill, abdomen and gut)

were gently washed in the sterile distilled water and dispersed in 100 ml sterile 'aged' seawter. Serial dilutions were prepared and streaked on Zobell 2216E medium[4] (composition given below) in duplicates. After 24-48h of incubation at 30°C (± 2°C), bacterial colonies were counted with the help of a Colony Counter. Besides the Zobell medium, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar[5], *Cytophaga* agar[4], 110 medium[6], and King's B medium[7] were also used for isolations of bacteria. Morpholgically distinct colonies were lifted and taken in culture tubes on Zobell 2216E medium. The cultures were purified by repeated streaking on agar plates to get single cell colonies. Discrete colonies from each plate were subcultuted and stored in a refrigerator. Standard diagnostic staining reactions, physiological and biochemical tests were performed for each isolate and identifications upto species level were made with the help of Bergey's Manual of Determinative Bacteriology[8] and Bergey's Manual of Systematic Bacteriology[6,9].

Media composition : Zobell 2216E medium (peptone 5.0g, yeast extract 1.0g, ferrous phosphate 0.01g, agar 15g, distilled water 250ml, aged seawater 750ml, pH 7.6–7.8), TCBS agar (peptone 1.0g, ferric citrate 0.1g, ox bile 0.8g, sodium citrate 1.0g, sodium thiosulphate 1.0g, sodium chloride 1.0g, sucrose 2.0g, yeast extract 0.5g, bromothymol blue 0.004g, thymol blue 0.004g, agar 1.4g, distilled water 25ml, aged seawater 75ml, pH 8.6), *Cytophaga* agar (tryptone 0.05g, yeast extract 0.05g, beef extract 0.02g, sodium acetate 0.02g, agar 0.9g, agd seawater 100ml, pH 7.2), Glycerin agar (glycerin 20g, glycin 2.5g, dipotassium hydrogen phosphate 1.0g, ferrous sulphate 0.1g, magnesium sulphate 0.1g, calcium carbonate 0.1g, agar 15g, aged seawater 100ml, pH 7.5), 110 medium yeast extract 2.5g, tryptone 10.0g, gelatin 30g, lactose 2.0g, mannitol, 10g, sodium chloride 5.0, agar 15g, distilled water 250 ml, aged seawater 750ml, pH 7.0- 7.2), King's B medium (peptone 20.0g, dipotassium phosphate 2.5g, magnesium sulphate 15g, glycerol 15ml, distilled water 250ml, aged seawater 750ml, pH 7.2).

Measurement and Assessment of Microbial Diversity

Frequency Index[10] : On the basis of percentage occurrence, the organisms were classified as *common* (> 10%), *frequent* (5–10%), *occasional* (1–5%) or *rare* (<1%). Shannon Index (H')[11] was calculated by the following formula:

$$H' = -\sum_{i=1}^{S} pi \ln pi$$

Where, s = the number of species
pi = the proportion of individual abundance of the i^{th} species
ln = log base e

Results and Discussion

The total bacterial counts (No. X 10/cm^2) from various parts (Table 1) varied with the season : between 10–65 for gill, 18–70 for carapace, 65–90 for gut and between 95–120 for abdomen. The counts were highest in monsoon/post-monsoon months and lowest in May.

Table 2 shows that the isolates were mostly, 15 out of 17, Gram- negative organisms; the Gram-positive bacteria were represented by *Bacillus subtilis* and *Staphylococcus albus.*

Table 1 – Total bacterial count (No. × 10^4/cm^2) from the various body parts of the fiddler crab.

Months	Gill	Carapace	Gut	Abdomen
Nov. 96	58	60	85	110
Jan. 97	63	60	76	115
Mar. 97	37	55	77	112
May 97	27	18	65	95
July 97	35	41	88	100
Sept. 97	49	70	90	120
Nov. 97	44	65	81	120
Jan. 98	65	64	89	118
Mar. 98	17	38	68	96
May 98	10	20	66	99
July 98	33	43	73	119
Sept. 98	46	68	70	119
Nov. 98	65	69	71	121

Table 2 – Frequency Index (FI) and percent occurrence (P) of bacterial isolates from various body parts of fiddler crab. (C= >10% F = 5-10%, O = 1-5%, R = < 1%).

Isolates	Gill		Carapace		Gut		Abdomen	
	FI	P	FI	P	FI	P	FI	P
Alcaligenes sp.	F	6.59	O	4.55	F	8.60	F	8.89
Bacillus cereus	F	7.69	F	6.82	O	3.23	F	5.56
B. subtilis	O	4.40	F	6.82	O	4.30	O	3.33
Brevibacterium sp.	O	3.30	O	3.41	F	5.38	F	5.56
Flavobacterium balustinum	O	3.30	O	2.27	–	–	–	–
Micrococcus sedenterius	O	4.40	C	11.36	C	13.97	C	16.67
M. roseus	–	–	F	6.82	F	8.60	F	7.78
Non fluorescent Pseudomonas sp.	*F*	*5.49*	*F*	*6.82*	*O*	*4.30*	*O*	*3.33*
P. aeruginosa	F	6.59	F.	5.68	O	2.15	O	3.33
P. fourescens	C	10.99	F	5.68	O	3.23	O	2.22
Serratia marcescens	F	9.89	O	3.41	F	7.53	–	–
Staphylococcus albus	F	5.49	O	4.55	F	8.60	F	8.89
Streptococcus sp.	F	7.69	C	10.23	F	8.60	C	11.11
Vibrio sp.	F	9.89	F	5.68	F	7.53	C	11.11
V. fischeri	O	4.40	F	5.68	O	3.23	–	–
V. fluvialis I	F	6.59	F	7.95	F	6.45	F	8.89
V. parahaemolyticus	O	3.30	O	2.27	O	4.30	O	3.33

Also most of the organisms were present on all part of the body except *Micrococcus roseus*, which was not found on gill. *Flavobacterium balustinum* were not found in gut and abdomen, and *Serratia marcescens* and Vibrio fischeri, which were not observed in abdomen.

Table 2 shows the Frequency Index and % occurrence of the identified bacteria. The extract data as summarized below sugest that vibrios and micrococci constituted greater

part of the bacterial population: the former on gill and carapace and the latter on internal organs viz. gut and abdomen. However, the gill did not support gorwth of micrococci, and pseudomionads were close to vibrios in % occurrence. On gut and abdomen also the difference in population size between micrococci and vibrios was not much which occurred with the other organisms. On carapace also both micrococci and pseudomonads were much less in number than the predominant vibrios.

Gill : *Vibrio* (24.21%) > *Pseudomonas* (23.09%) > *Bacillus* (12.09%)

Carapace : *Vibrio* (21.58%) > *Micrococus* (18.18%) = *Pseudomonas* (18.18%)

Abdomen : *Micrococcus* (24.45%) > *Vibrio* (23.33) > *Pseudomonas* (11.11%)

Gut : *Micrococcus* (22.57%) > *Vibrio* (21.51%) :: *Pseudomonas* (9.47%)

The Frequency Index (FI) data (Table 2) suggest that the 17 bacterial isolates ranged between *common* to *occasional*, maximum number of isolates were *frequent* (6–9) while 5-7 were *occasional.* None was *rare* at any body part. The FI of the bacterial isolates (number) on each part is summarized below:

	common	frequent	occasional	rare
gill	1	9	6	Nil
carapace	2	9	6	Nil
gut	1	8	7	Nil
abdomen	3	6	5	Nil

For the total isolates (91 from gill, 88 from carapace, 93 from gut and 90 from abdomen), Shannon's index value for gil, carapace, gut and abdomen is 2.707, 2.750, 2.664 and 2.487, respectively, indicating carapace as the most and abdomen as the least diverse habitat.

Our study of bacteria associated with fiddler crab indicates, the dominance of Gram-negative bacteria. Among the bacteria identified, 10 genera were from gill and carapace, 9 were from gut and 8 were from abdomen. Great diversity was found on carapace. Genus *Flavobacterium* was recorded only in gill and carapace samples. Distribution of *Serratia* was restricted to gill, carapace and gut samples.

On gill and carapace, vibrios outnumbered others. *Pseudomonas* was next to *Vibrio* in gill samples, followed by bacilli, while, in carapace samples *Micrococcus* was at second place followed by *Pseudomonas.* This is in agreement with reports of Abhay Kumar[12].

In gut and abdomen samples, *Micrococcus* outnumbered *Vibrio* and *Pseudomonas.* Abhay Kumar[12] has also reported similar results. Venkateswaran *et al.*[13], reported *Micrococcus* as the dominant organism in the gut of some edible crabs found in Portonovo coast.

The organisms from various parts may be beneficial in terms of nutrition by providing vitamins and enzymes which contribute to the digestion of complex food materials[14,15].

Like us, Jayseela Fatima *et al.*[16], have also found a high population of *Vibrio* in the stomach of *Rastralliger kanagurta.* Mary *et al.*[17], recorded a high population of vibrios in the gut of certain estuarine fishes including mudeater. In our study, among vibrios, *Vibrio* sp. and *V. fluvialis* I were dominant. *V. fischeri.* was not recorded in abdomen samples. Besides vibrios, micrococci and pseudomonads, *Bacilus, Alcaligenes,* etc. have also been found from different parts. Mary[18] has reported these organisms in mullet. *Bacillus cereus, Alcaligenes, Pseudomonas fluorescens* have been included in surface microflora and *Flavobacterium balustinum* is considered to be a fish pathogen[1].

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Relative absorbency of some trace elements by various forms of macrophytes in a tropical water body

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Abstract

During the present study it has been found that some trace elements like copper, zinc and cadmium are absorbed by floating macrophytes while lead and manganese is trapped in higher quantities by submerged and floating plants. On the other hand emergents have been found to be less efficient compared to floating and submerged plants.

(**Keywords:** trace elements/macrophytes)

Introduction

Heavy metals are non bio-degradable, persistent in nature for a long period, toxic to living organisms at fairly low concentrations and tend to either biologically magnify or accumulate in plants and animal systems.

It is known that aquatic plants can be used for the removal of toxic metals from polluted waters[1,2,3]. Aquatic plants can accumulate nutrients from a concentration gradient, serving as indicators of nutrient availability in aquatic environments[4,5,6] and also of heavy metals and rare earth elements[1,5]. The input of trace elements to freshwaters are increasing which often results from industrial and combustion emissions, which are subsequently deposited onto the drainage basins of rivers and lakes[7].

The very purpose of this study was to evaluate the effectiveness of different types of macrophytes in trapping trace elements.

Place of work : The present work was conducted on an important vulnerable, potable water resource approximately 5km away from the main Bhopal city railway station, lying at an altitude of 494 m.a.s. 1, within the geographical coordinates of 23°10'-23°20'N and 77°15' - 77°25'E. It is the chief source of potable water for the local residents of Bhopal. Unfortunately this water body has been threatened to a great extent, due to accelerated drainage, land reclamation and increase in the human activities in its catchment area (361 sq. km.).

Materials and Methods

At upper lake, sixteen stations were selected in the littoral zone in the present study.

Physicochemical parameters viz., temperature, pH, conductivity, dissolved oxygen, total dissolved solids etc., were analysed in the field itself as they are liable to changes. The methods followed for water chemistry are as given in different books[8,9,10]. For the extimation of heavy metals the samples (macrophytes) were digested by diacid digestion, then allowed to cool and filled upto 100ml with double deionised water, metal concentrations were determined by AA technique[11].

Macrophytes for qualitative and quantitative analysis were collected using a wooden quadrate of 1 m^2. After sorting out species wise (care was taken to blot out excess water), they were weighed to record the fresh weight.

For the estimation of heavy metals the water samples were treated with concentrated nitric acid for further analysis on Atomic Spectrophotometer[11].

Results

Important physico-chemical characteristics of present water body has been summed up in Table 1.

Table 1 – Some physico-chemical parameters of the water body (yearly average).

Months	Temp. (°C)	pH (mg/l)	D.O. (mg/l)	T.H. (mg/l)	T.D.S. (mg/l)	Alkalinity (mg/l)	Chloride (mg/l)	Nitrate (mg/l)	T.P. (mg/l)	Orthophosphate (mg/l)
January	21.1	8.1	8.3	110.1	127.6	58.35	22.9	0.26	0.32	0.06
February	21.4	8.4	8.4	77.2	152.5	83.5	19.7	0.28	0.25	0.06
March	28.3	7.3	3.9	87.4	130.5	91.4	21.3	0.22	0.21	0.08
April	29.5	7.3	6.6	79.8	135.7	106.2	21.3	0.23	0.21	0.07
May	28.3	7.5	4.8	82.9	105.7	86.6	26.2	0.22	0.21	0.08
June	30.2	7.9	6.2	89.2	97.9	96.3	21.5	0.21	0.21	0.07
July	27.1	8.2	6.2	90.8	127.3	87.6	32.3	0.47	0.36	0.14
August	27.4	7.3	6.6	89.6	109.5	81	33.2	0.54	0.38	0.13
September	24.8	8	5.6	88.3	90.85	83.8	29.3	0.21	0.32	0.07
October	25	8.2	6.3	86.3	133.85	80	29.4	0.22	0.32	0.06
November	21.1	7.6	6.1	66.15	119.05	77.1	29.9	0.26	0.25	0.05
December	20.6	7.6	6.3	117.4	113	74	15.5	0.39	0.38	0.04

On the basis of seasonal percent cover of macrophyte as recorded in Table 2, the following yearwise trend was discernable:

1995–97 : Emergent > Submerged > Floating

The macrophytic cover varies seasonally with the change in atmospheric condition, Table 2 and Fig. 1. Thus, emergents have been found to be the dominant macrophytes occupying maximum lake area under present condition. Further, it was recorded that during the span of 3 years, with the change in yearly climatic pattern, an increase in macrophytic coverage from '95 to 97' during summer months has been to the tune of 22%, while during postmonsoon macrophytes recorded an increase by 3% (Table 3).

Table 2 – Percent change in area covered by aquatic vegetation.

Year	Submerged				Floating				Emergent			
	S	Pm	W	Avg.	S	Pm	W	Avg.	S	Pm	W	Avg.
95-96	32	39	36	36	17	2	7	9	50	69	57	59
96-97	47	ND	21	34	19	ND	32	26	34	ND	47	41
97	26	14	ND	20	21	10	ND	16	53	75	ND	64

Table 3 – Change in macrophytic coverage (sq. km. & in %).

Year	Summer		Post Monsoon		Winter	
	sq. km.	%	sq. km	%	sq. km.	%
95-96	2.915	11.64	3.402	13.58	6.275	25.05
96-97	6.21	24.82	ND	ND	4.9	19.56
97	8.47	33.8	4.648	18.56	ND	ND

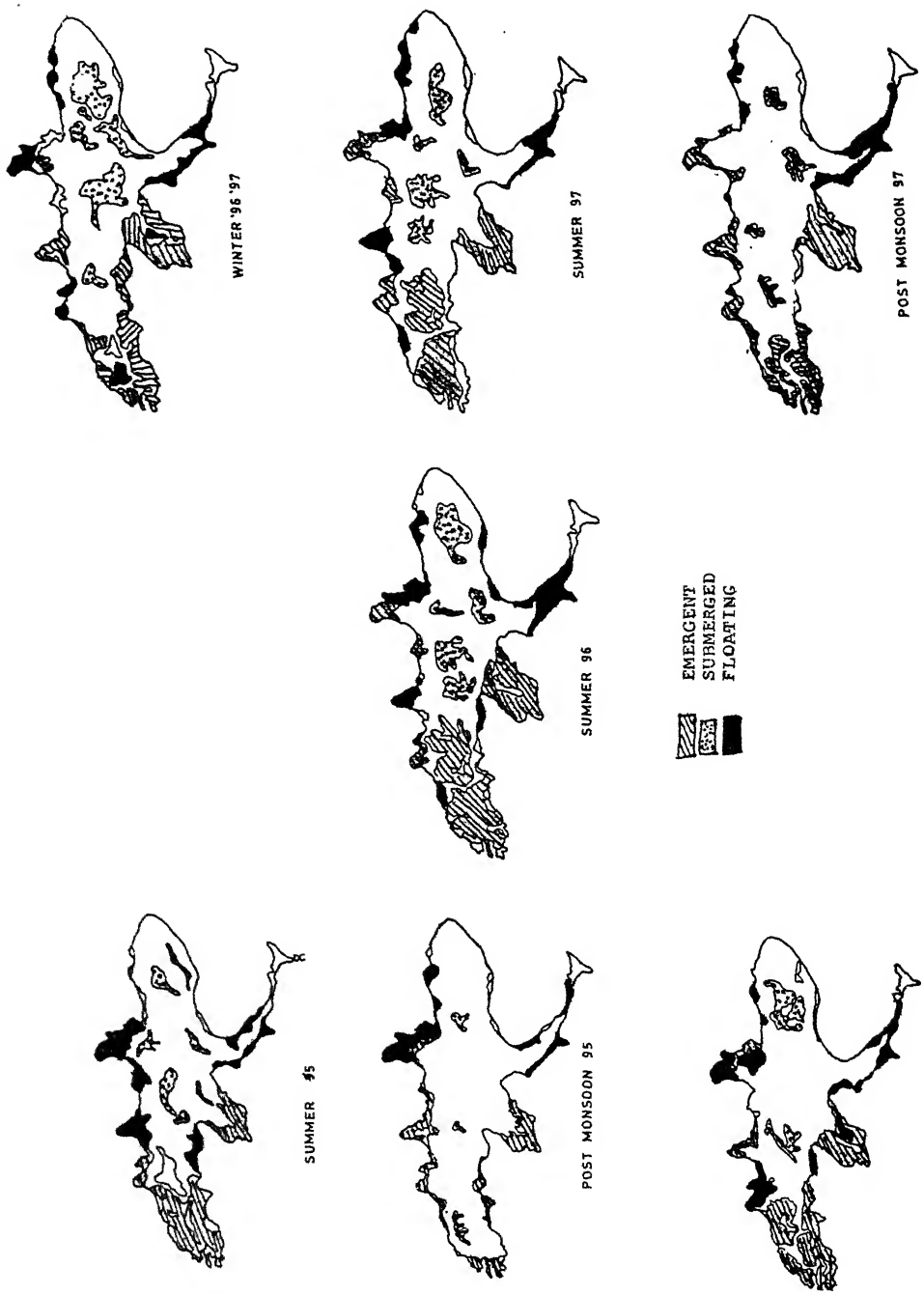

Fig. 1 – Seasonal macrophytic coverage in the study area (95-97).

Table 4 records average fresh weight of macrophytes during the three year study period. The contribution of each form of macrophytes was found to be varying with varying dominance of individual macrophytic species of the group from year to year.

During 1995-96 summer, at station VIII amongst emergent macrophytes maximum (2408.4 gm/m^2) contribution was recorded by *Typha latifolia* (53%). *Ceratophyllum demersum* (17%) and *Hydrilla verticillata* (26%) contributed maximum share towards total bio-mass (2835.4 gm/m^2) at station III amongst the submerged group of macrophytes. *Eichhornia crassipes* represented the floating macrophyte which contributed about 8669.6 gm/m^2 (84%) (Table 4).

During summer of 1996, emergent macrophytes recorded maximum contribution (3951.1 gm/m^2) at station VIII (*Polygonum glabrum*, 41% and *Typha latifolia*, 20%) while station IX recorded maximum (2827.4 gm/m^2) contribution of *Potamogeton pectinatus* (31%), *Potamogeton nodosus* (36%), and *Hydrilla verticillata* (33%) which represented the submerged group of macrophytes. Among floating macrophytes, *Eichhornia crassipes* (76%) noted maximum contribution (2270.5 gm/m^2) at station X (Table 4).

Table 4 – Change in fresh weight (gm/m^2) of various macrophytes both seasonally and yearwise.

Year	Emergent	Submerged	Floating
Summer			
95-96	2408.4	2835.4	86696
96-97	3951.1	2827.4	2270.5
97	3745.9	2332.8	1719.5
Post Monsoon			
95-96	1509.3	1456.7	1259.6
96-97	ND	ND	ND
97	1508.4	1496.3	872.1
Winter			
95-96	1606.5	4088.2	3160
96-97	3538.9	3668.7	2331.2
97	ND	ND	ND

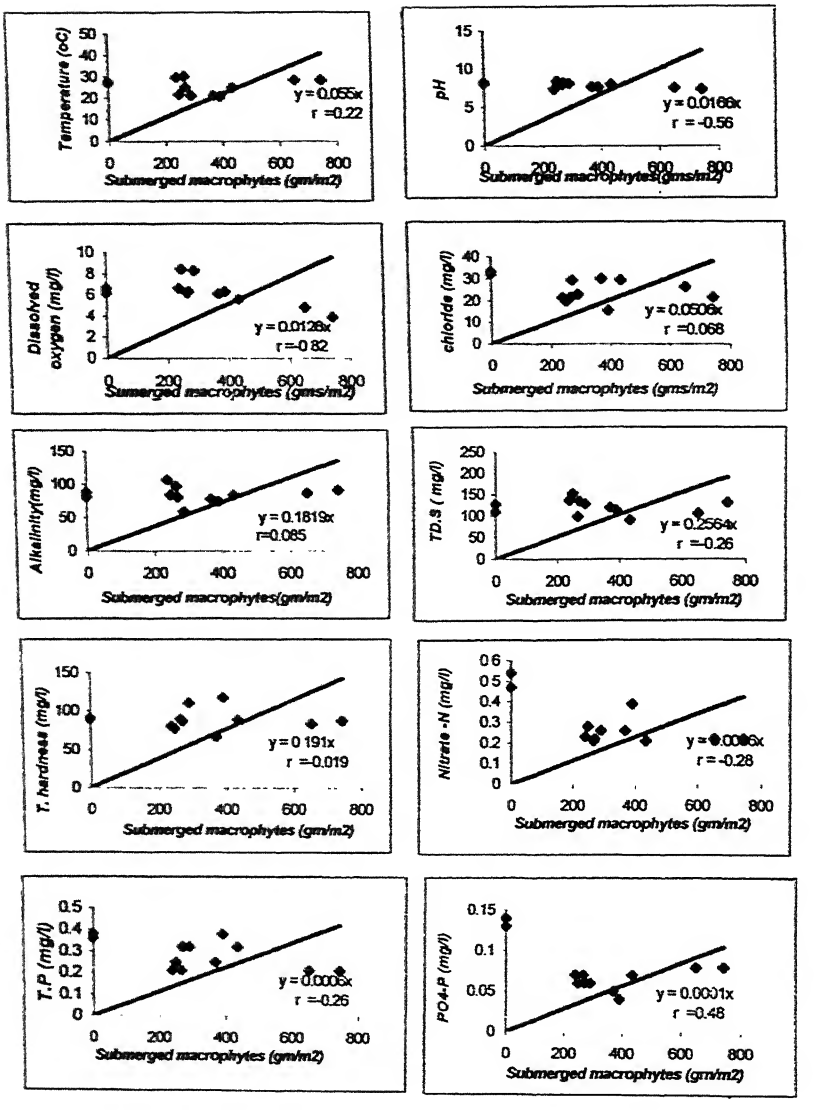

Fig. 2 – Correlation co-efficients between physico-chemical parameters and Submerged macrophytes.

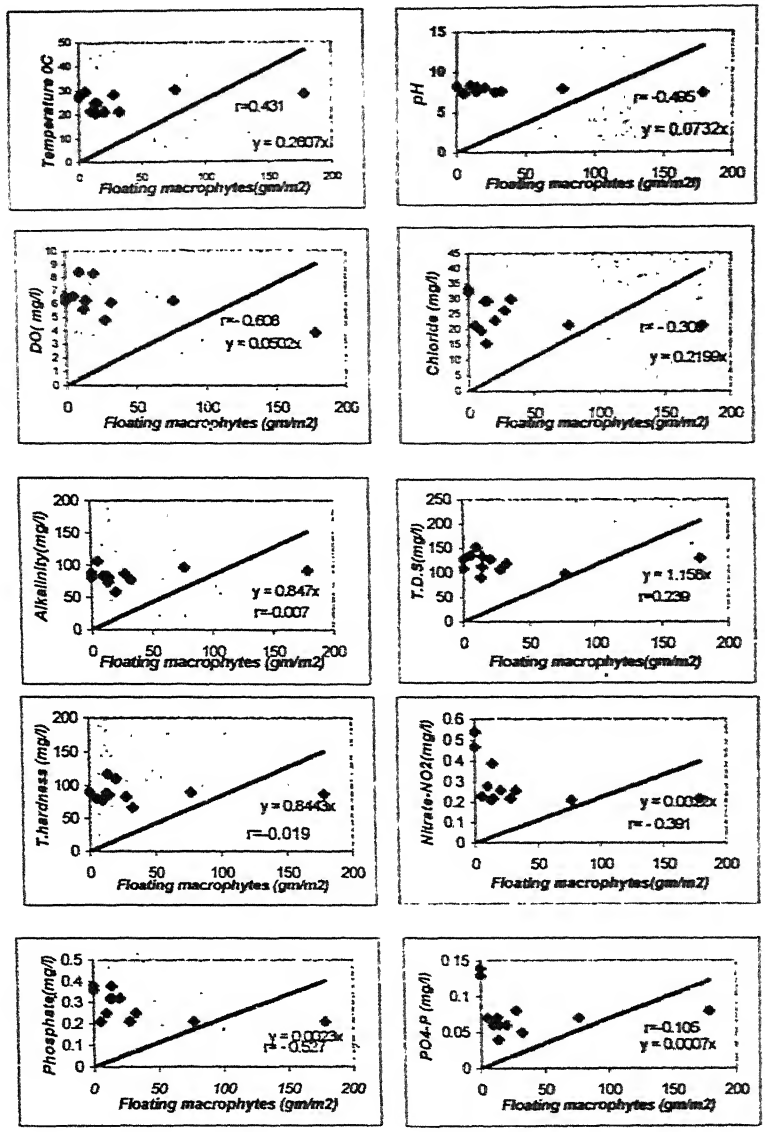

Fig. 3 – Correlation co-efficients between physico-chemical parameters and floating macrophytes.

During summer months of 1997, the maximum contribution (3745.9 gm/m^2) of emergent macrophytes (*Polygonum glabrum* - 38%, *Typha latifolia* - 17% and *Scirpusroyle* -16.15%) was recorded at station X. Submerged macrophytes (*Potamogeton pectinatus* 32.3%, *P. nodosus* 30.4% and *Hydrilla verticillata* 33.5%) recorded maximum contribution (2332.8 gm/m^2) at station IX while *Azolla pinnata* and *Trapa bispinosa* contributed maximum (1719.5 gm/m^2) floating vegetation with 36% and 38% at station II respectively.

During postmonsoon season 1995, *Ipomoea aquatica* contributed over 50% of total (1509.3 gm/m^2) emergent group at station VIII. Maximum contribution (1456.7 gm/m^2) of submerged macrophytes marked at station X by following species: *Potamogeton pectinatus* (21%), *P. natans* (17%) and *Hydrilla verticillata* (17%). Among floating macrophytes, maximum contribution (1259.5 gm/m^2) was recorded at station II with 82.6% contribution of *chhornia crassipes* (Table 4). Postmonsoon ('97) season recorded maximum contribution (1508.4 gm/m^2) of emergent macrophytes at station VIII. Of the submerged vegetation, (1496.3 gm/m^2) *Potamogeton pectinatus* and *Myriophyllum spathulatum* contributed 24.2% and 26.4% respectively at station I while amongst floating macrophytes, *Eichhornia crassipes* contributed (872.1 gm/m^2) about 91% at station II.

Winter 1995-96 marked maximum contribution of emergent (1606.5 gm/m^2) and submerged vegetation (4088.2 gm/m^2) at station VIII and station IX with *Ipomoea aquatica* (24.2%), *I. fistulosa* (19%), *Typha latifolia* (27.2%) and *Potamogeton pectinatus* (34.9%), *P. nodosus* (26%), *Hydrilla verticillata* (27.8%) respectively (Table 4). Among floating macrophytes maximum (3160 gm/m^2) was recorded at station II with 82.6% contribution of *Eichhornia crassipes.* During winter (1996-97) season, the maximum contribution (3538.9 gm/m^2) of emergent vegetation was noted at station VIII with *Typha latifolia* and *Polygonum glabrum* 24% and 26% respectively. Among submerged macrophytes, *Potamogeton pectinatus* (29.1%), *P. nodosus* (27%) and *Hydrilla verticillata* (27.1%) contributed maximum (3668.7 gm/m^2) at station II. Of the floating ones, *Eichhornia crassipes* contributed 2331.2 gm/m^2 (56%) at station X (Table 4).

Relationship between macrophytic abundance with some physico chemical parameters : The presence of various macrophytes under varying chemical characteristics in a tropical water body is defined in Table 5, while the correlation co-efficients derived between various physico-chemical parameters and macrophytes have been reported in Fig. 2,3.

Change in trace element concentration in different macrophytes : Hyrilla verticillata absorbed maximum concentration of copper with 0.05 mg/100 gm of all other trace elements during the present study (Table 6). *Myriophyllum spathulatum* absorbed 0.135 mg/100 gm of manganese which was the highest. *Eichhornia crassipes* absorbed 0.702 mg/100 gm of copper while 0.326 mg/100 gm of copper was absorbed by *Potamogeton nodosus.*

Table 5 – Plant distribution in relation to water chemistry.

MACROPHYTES	CHEMICAL CHARACTERISTICS							
	pH	T. hardness mg/l	T.D.S. mg/l	Alkalinity mg/l	Chloride mg/l	NO3-N mg/l	PO4-P mg/l	RA, RPB (1968) Present study
Potamogeton crispus	7.5-8	100-350	Pc	170-250	66-1000	< 0.3	< 0.3	RA, RPB (1968)
	7.3-8.3	66-117	90-153	58-106	15.5-33.2	0.21-0.54	0.21-0.38	Present study
Potamogeton natans	Pc	100-350	100-350	170-250	66-1000	0.03	0.03	RA, RPB (1968)
	7.8-8.3	66-117	90-153	58-106	15.5-33.2	0.21-0.54	0.21-0.38	Present study
Potamogeton pectinatus	8	100-350	> 350	170-250	100	6	0.3-1.2	RA RPB (1968)
	7.8-83	66-117	90-153	58-106	15.5-33.2	0.21-0.54	0.21-0.38	Present study
Ceratophyllum demersum	Pc	100-350	100-350	170-250	100	0.3-6	0.31-1.2	RA, RPB (1968)
	7.8-8.3	66-117	90-153	58-106	15.5-33.2	0.21-0.54	0.21-0.38	Present study
Myriophyllum spathulatum	7.5-8	100-350	< 100	170-250	Pc	< 1	3	RA, RPB (1968)
	7.8-8.3	66-117	90-153	58-106	15.5-33.2	0.21-0.54	0.21-0.38	Present study

Pc-poorly correlated

Table 6 – Change in trace element concentration in individual macrophytes (mg/100gm).

Individual Macrophytes	Copper	Zinc	Lead	Manganese	Cadmium
Hydrilla verticillata	0.059	0.01	0.007	0.021	0.005
Myriophyllum spathulatum	0.018	0.11	0.091	0.135	0.048
Eichhornia crassipes	0.702	0.36	0.003	0.156	0.073
Potamogeton nodosus	0.326	0.093	0	0.018	0.01
Ceratophyllum demersum	0.131	0.068	0.003	0.02	0.002
Ipomoea aquatica	0.511	0.154	0.005	0.016	0.037
Polygonum glabrum	0.275	0.106	0.009	0.027	0
Vallisnaria spiralis	0.06	0.116	0.005	0.016	0.004
Najas major	0.04	0.244	0.016	0.031	0.01
Nymphoides cristatum	1.21	0.4	0.012	0.016	0.007
Azolla pinnata	0.187	0.57	0.007	0.027	0.033

Ceratophyllum spathulatum, Ipomoea aquatica, Polygonum glabrum, Nymphoides cristatum and *Azolla pinnata* absorb 0.131mg/100gm, 0.511mg/100gm, 0.275mg/100 gm, 1.21mg/100gm and 0.187mg/100gm of copper respectively which is in higher values of all the other trace elements. *Vallisnaria spiralis and Najas major* absorbed 0.116mg/100gm and 0.244mg/100gm of zinc (Table 6).

Out of all the sites, the maximum concentration of copper was observed at station X in composite macrophytes while station II recorded maximum values (0.870mg/100gm and 0.728 mg/100 gm) of zinc and manganese respectively. A maximum of 0.026 mg/100 gm of lead was recorded at station V while at station VI 0.050 mg/100 gm of cadmium was absorbed by the composite macrophytes (Table 7).

Table 7 – Change in trace element concentration in composite macrophytes (mg/100gm)

Station	Copper	Zinc	Lead	Manganese	Cadmium
I	0.043	0.42	0.011	0.294	0
II	0.027	0.87	0	0.728	0.008
V	0.027	0.483	0.026	0.288	0.038
VI	0.056	0.253	0.005	0	0.05
VII	0.072	0.847	0	0.549	0.015
VIII	0.043	0.53	0	0.002	0.007
IX	0.043	0.603	0.02	0.318	0.018
X	0.271	0.703	0.005	0.323	0.004
XI	0.043	0.727	0.013	0.031	0.011
XII	0.04	0.445	0.003	0.345	0.05
XIII	0.024	0.335	0	0.305	0.04
XIV	0.022	0.535	0	0.22	0.045
XV	0.017	0.384	0.007	0	0.03
XVI	0.044	0.69	0.001	0	0

Discussion

Around 77% of the lake area is weed infested, out of which submerged weeds contribute around 42% while emergent and floating weeds contributed almost equal percentage towards the lake area (29% each). Since the present water body is shallow in nature, macrophytic vegetation through out depends upon the morphometry, light and its slope. The distribution of emergent macrophytes depends upon the average slope and morphometry of the lake while the distribution of submerged plants mainly depends upon the under water light[12]. The zonation of macrophytes depends upon the wave action, sediment and light climate[13].

The plants have been found to accumulate nutrients besides processing them into various forms depending upon their individual capacities with respect to the trace elements entering into the system from anthropogenic sources, it has been found that macrophytes of different types accumulate them in different proportions. On observing the lock up mechanism of nutrients, by macrophytes, it has been found that with respect to the inlet source and the dominance of specific communities responsible for trapping the nutrients brings in variation in the "sequence of dominance" of trapping the trace elements.

Maximum absorption of copper was observed in *Nymphoides cristatum* (1.25 mg/100 gm) while that of zinc was seen in *Eichhornia crassipes* (0.36 mg/100 gm), *Najas major*

(0.244 mg/100 gm) and *Nymphoides cristatum* (0.400 mg/100 gm). Manganese was observed in higher values (0.135 mg/100 gm) by *Myriophyllum spathulatum. Eichhornia crassipes* was found to be the most efficient macrophyte. It absorbed copper (0.702 mg/100 gm) zinc (0.36 mg/100 gm), manganese (0.156 mg/100 gm) and cadmium (0.073 mg/100 gm).

As far as individual species are concerned the quality of water is to be assessed and the species usually occur in wide range of habitats not well related to individual nutrients of the water. Nutrients are one of the complex factors determining plant distribution and other factors as depth, morphometry, rock and soil type, seasonal fluctuations are also responsible for the same. In controlling the distribution of plants, nutrients and water movements play an important role[2]. The hardness ratio is the combined calcium and magnesium present in the site divided by the sodium and potassium[14] and has proved very useful in interpreting lake vegetation in terms of nutrient status for plants. Both ratios and quantities of nutrients are important in determining how plants react to the chemical regime.

The concentration of zinc, manganese, copper, cadmium and lead recorded maximum value of 0.870 mg/100 gm, 0.728 mg/100 gm, 0.271 mg/100 gm, 0.050 mg/100 gm and 0.026 mg/100 gm respectively in composite macrophytes of different inlets. Thus proving the efficiency of macrophytic communities in locking up of nutrients in different inlets.

On yearly average basis, following efficiencies of trapping trace elements at different inlets by different types of macrophytes have been arrivd at:

COPPER mg/100gm/yr	Floating > (0.69)	Emergents > (0.041)	Submerged (0.105)
ZINC mg/100gm/yr	Floating> (0.272)	Emergents > (0.123)	Submerged (0.106)
LEAD mg/100gm/yr	Submerged > (0.020)	Floating > (0.007)	Emergents (0.0025)
MANGANESE mg/100mg/yr	Floating > (0.066)	Submerged > (0.040)	Emergents (0.017)
CADMIUM mg/100gm/yr	Floating > (0.037)	Emergents > (0.023)	Submerged (0.013)

Under present investigation, free floating macrophytes have been found to be efficient in trapping most of the heavy metals in comparison to submerged and emergent macrophytes. This may be on account of the fact that free floating macrophytes generally assimilate nutrients from the water. Free floating macrophytes have been observed to have greater

metal removal efficiencies[15]. Heavy metals (Zn, Cr, Cd) were totally depleted from the nutritive solution suggesting complete absorption by water hyacinth[16]. On the other hand, submerged and rootless macrophytes (viz. *Ceratophyllum)* take up elements directly from water due to reduced structure and vascular system besides, through their finely divided leaves (*Ceratophyllum*[6,10,17]).

The presence of nutrients in ambient waters does not show any timely significant relationship with macrophytes. The reason being that once the nutrients reach the water their uptake is either simultaneously[18] (luxuriant uptake) or at a time when optimal environmental conditions return. This is why during monsoon periods high runoff, bring in good load of nutrient from the catchment area does not show enhancement in the macrophytic growth. However, when the atmospheric temperature/water temperature become congenial during post monsoon period an outburst in the macrophytic growth/ accumulation of macrophytic biomass has been observed. Since the aquatic resource forms dynamic systems which are always in a state of flux as such, significant relationships between individual chemical parameter and macrophytes has not been deciphered. Though on seasonal basis a time lag trend in growth of macrophytes has been observed.

On comparing present study with the river authorities[19-21] reveal that there is no good deal of correlation between the water nutrients and that of the aquatic plants (Table 5). According to River authorities[19-21], when plants are correlated with the levels of chloride, nitrate and total dissolved solids, the correlation is not so good and in the groups with common requirements (for alkalinity, pH and water hardness), also there is no such relation. Water nutrients cannot account all trophic variations between species.

Depending upon the plant species and type of water body macrophytes may accumulate nutrients,thus decreasing their concentrations in water. However, it is important to note that the protective function of the ecotonal region of the lake is limited with increasing pollution as long term influence of increased pollution load results in elimination of macrophytes and associated (microflora and micro-macro fauna) insects. Macrophytes cannot play a significant role in the nutrient entrapment in waters with high concentrations of pollutants[18].

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Impact of trace metals on the primary productivity of brackishwater ecosystem of Sundarbans, West Bengal

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Abstract

Bioassay experiment to determine the *in situ* impact of trace metals (Cu, Zn, Pb and Cd) addition at different concentrations (5-4,000 $\mu g\ l^{-1}$) on the primary productivity of a sewage mixed brackishwater canal was evaluated. The efficiency of toxicity of the studied metals was observed to be in the following order : Cu>Cd>Zn>Pb. The median effective concentration on both gross primary production and respiration was determined. Lead was observed to have no impact on community respiration.

(Keywords : impact/trace metals/primary production).

Introduction

Fixation of carbon through primary production is the main source of energy for organisms in all trophic levels in aquatic ecosystem. The ability of natural waters to support the level of primary production expected from ambient light and nutrient conditions depends upon maintaining the available forms of every metal at a concentration between toxicity and deficiency. Primary production is generally influenced by different essential metals *e.g.,* Cu, Zn, Fe, Mn, Co, etc. and toxic metals e.g., Hg, Pb, Cd etc.[1-2]. In natural waters, some metals such as Cu and Zn can also act either as a stimulatory or inhibitory way, depending on their speciation and availability in the environment[3]. Agricultural, industrial and domestic wastes are the main source of trace metals in aquatic ecosystems. Effluent comprising trace metals discharged from these sources into the aquatic systems may pose a serious problem of the system through adversely affecting the phytopankton production and other aquatic biota. Scanty information exists[4-9] as regards the effect of trace metals on the phytoplankton production. In view of this fact, attempt has been made to study the

impact of some trace metals on the primary productivity of a lotic brackishwater habitat having very high aquacultural importance.

Study area : The study was conducted in the tidal water of Jagannath canal near the Goberia Abad flood plain of Haroa, North Sundarbans, West Bengal (Fig. 1). The canal is connected to the Bidyadhari River near Kulti. The large quantity of sewage of Calcutta metropolis is being mixed with the brackishwater of Bidyadhari River and flows northeast through the Jagannath canal due to tidal pressure[10]. As reported by Khan[11], some of the physicochemical characteristics of sewage before mixing with brackishwater are : pH, 7.5-7.8; total solids, 820.4-2150.2 mgl^{-1}; dissolved oxygen, 0 mgl^{-1}; total alkalinity, 219.2-265.5 mgl^{-1} as $CaCo_3$; BOD_5, 20.3-65.5 mgl^{-1}; ammonia, 4.3-14.5 mgl^{-1}; nitrate, 2.1-4.5 mgl^{-1}; phosphate, 3.0-12.5 mgl^{-1}. Considering this sewage mixed brackis water beneficial for aquaculture, an estimated 3,600 ha shrimp/fish culture bheries have developed along the two banks of the canal and its arteries. The process of developing these bheries have been started in the mid 80's[12]. In the rainy season when water becomes almost fresh, most of these bheries have been used for paddy cultivation. The people of this area are directly or indirectly dependent on these bheries for their subsistence.

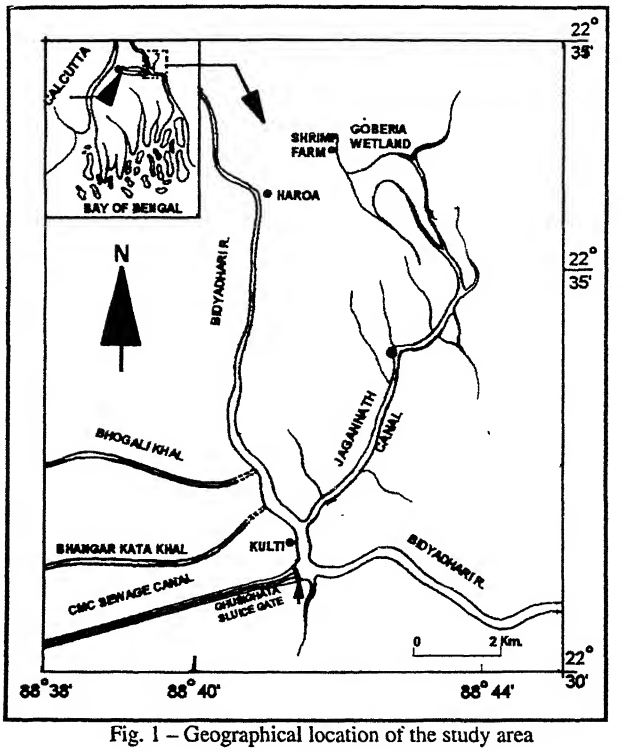

Fig. 1 – Geographical location of the study area

Materials and Methods

The concentrations of various trace metals used in the experiment are furnished in the Table 1. The experiment was conducted by following the method as described by Goldman[13]. The primary productivity was determined by the classical light and dark bottle technique[14] and the dissolved oxygen (DO) was determined by the modified Winkler's titrimetric method. Stock solution of each metal was prepared by dissolving analytical grade salts in

Table 1 – Concentrations of different trace metals used in the experiment.

Metals	Source	Concentrations (μg l^{-1})
Copper	$CuSO_4$, $5H_2O$	
Zinc	$ZnSO_4$, $7H_2O$	5, 10, 50, 100, 500,
Lead	$Pb(NO_3)_2$	1000, 2000 & 4,000
Cadmium	$CdCl_2$	

Table 2 – The average physicochemical characteristics of water of the study area of Jagannath canal during the study period.

Parameters	Quantity	Parameters	Quantity
Temperature (°C)	29.6	PO_4-P (μg-at l^{-1})	3.35
pH	7.93	SiO_2-Si (μg-atl^{-1})	129.54
Salinity (ppt)	2.02	Calcium (mg l^{-1})	50.16
Transparency (cm)	47.5	Magnesium (mg l^{-1})	89.12
Dissolved oxygen (mg l^{-1})	6.86	Copper (μg l^{-1})	32.58
Total alkalinity (mg l^{-1} as $CaCO_3$)	169.70	Zinc (μg l^{-1})	96.30
NO_3-N (μg-at l^{-1})	65.13	Lead (μg l^{-1})	7.56
NO_2-N (μg-at l^{-1})	6.51	Cadmium (μg l^{-1})	2.11
NH_4-N (μg-at l^{-1})	7.35		

Table 3 – EC_{50} (μg l^{-1}) value of trace metals of productivity of Jagannath canal.

Metals	Gross primary production (GPP)		Respiration (R)	
	*AC	**TC	AC	TC
Copper	116.16	185.46	19363	273.12
Zinc	548.28	767.49	3,609.11	3,358.87
Lead	5,592.62	5,351.67	–	–
Cadmium	207.69	212.86	1,441.12	1,433.45

*Considering added concentration; **Considering total concentration; '-' means no effect.

double distilled water. Working solutions were prepared in such a way so that every bottle (300 ml BOD bottles) can receive almost equal volume of solutions. Addition of working solutions to the bottles caused insignificant change of pH and salinity in the bottles. The physicochemical parameters of water were analyzed using standard methods[15-16]. The dissolved trace metals were analyzed by following the method outlined by Chakraborti *et al.*[17] The counting of phytoplankton was done by the direct census method[18] and the planktonic forms were identified using standard keys[19- 20]. The medium effective concentration (EC_{50}) value of trace metals on primary production was computed through probit analysis of Finney[21]. Total concentration of trace metals *i.e.*, ambient concentration and the added concentration were considered in computation of EC_{50} value.

Results and Discussion

The physicochemical characteristics of water of Jagannath canal during the study period were furnished in Table 2. The mean temperature of water was 29.6°C. The water of the canal was alkaline in nature. Total alkalinity was sufficient enough to support primary production. Concentrations of different nutrients were relatively quite high, indicative of sewage polluted condition of the ecosystem. The phytoplankton population, which was involved in primary production, was 10,125-10,350 nos l^{-1}. The phytoplanktonic forms were composed of species like *Schroederia* (7.50%), *Pediastrum* (10.30%), *Tetrastrum* (5.20%), *Scenedesmus* (8.74%), *Closterium* (6.52%), *Cyclotella* (2.20%), *Coscinodiscus* (5.42%), *Asterionella* (6.60%), *Navicula* (4.32%), *Melosira* (9.20%), *Nitzschia* (4.36%), *Anabaena* (10.66%), *Anabaenopsis* (8.70%), *Spirulina* (6.60%) and *Oscillatoria* (3.68%). Most of the planktonic forms are indicative of freshwater condition, which was also supported by Saha *et al.*.[22] The primary production (GPP) was 0.055-0.056 mgC l^{-1} h^{-1}

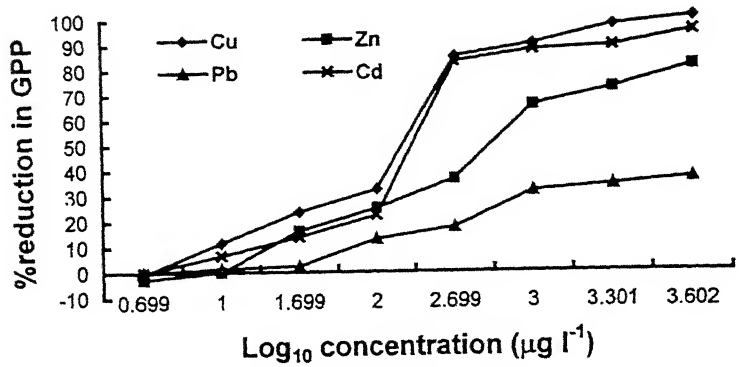

Fig. 2 – Effect of trace metals on the gross primary productivity (GPP) of phytoplankton of Jagannath canal, West Bengal.

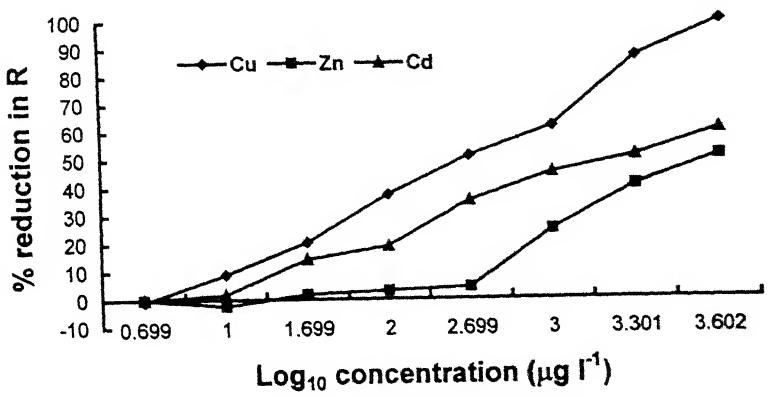

Fig. 3 – Effect of trace metals on the respiration (R) of phytoplankton of Jagannath canal, West Bengal.

with a community respiration (R) of 22.16-23.24% over GPP. The effect of trace metals on GPP and R has been depicted in Figs. 2 and 3, respectively. It is evident from the figures that the ambient concentration (μg l^{-1}) levels of all the four trace metals *viz.*, Cu (32.58), Zn (96.30), Pb (7.56) and Cd (2.11) were not toxic for the primary productivity of Jagannath canal. Moreover, the initial added concentration *i.e.*, 5.0 μg l^{-1} of Cu and Zn increased the GPP by 0.73% and 2.75% and R by 0.81% and 0.00% over control respectively. Addition of Pb and Cd showed no effect on both GPP and R at this concentration. Minimum inhibition of both GPP and R was observed when the ambient concentrations of Cu, Zn, Pb and Cd were increased to 42.58, 146.30, 17.56 and 12.11 μg l^{-1} respectively. From the EC_{50} value (Table 3), it is observed that copper was the most toxic (EC_{50} = 185.46 μg l^{-1}) and Pb was the least toxic (EC_{50} = 5,351.67 μg l^{-1}) among the studied metals. Considering EC_{50} values of the added concentrations, the toxicity of Cu was found to be 1.79, 4.72 and 48.15 folds higher than that of Cd, Zn and Pb, respectively. At the added concentration of 4,000 μg l^{-1} of Cu, both GPP and R values were found to be completely ceased within four hours of incubation (Figs. 2 & 3). Thus the magnitude of toxicity of the studied metals on the GPP of Jagannath canal was according to the hierarchy : Cu>Cd>Zn>Pb. The order of toxicity of trace metals on respiration followed the same trend as on GPP. Lead was observed to exert no impact on respiration even at the highest added concentration.

Most of the earlier workers did not consider the ambient level of metals during computation of EC_{50} value significant in determining the exact toxic concentration. However, Jindal and Verma[5] reported the order of toxicity of metals to primary production as : Cu>Cd>Pb>Zn and the EC_{50} values of Cu, Cd, Pb and Zn were 196.96, 3,078.00, 20,852.00 and 1,06,129.00 μg l^{-1}, respectively in a freshwater pond at Khuda Ali Sher (Chandigarh). In the saline environment of Mooringanga river, the order of toxicity of these four metals to primary production was represented as : Cu>Zn>Cd>Pb and the EC_{50} values of these four metals were 257.37, 320.57, 503.89 and 557.28 μg l^{-1}, respectively[9]. In Jagannath canal, toxicity of Cu was also highest with lower EC_{50} value (116.16 μg l^{-1}). But EC_{50} values of other metals were higher than those of Mooriganga River and lower than those of the freshwater pond. The EC_{50} value of Zn on the primary productivity of Jagannath canal was observed to be lower (767.49 μg l^{-1}) than that reported by Patrick[23], who recorded EC_{50} value of Zn on the production of a *Navicula* sp. which varied from 1,300 to 4,050 μg l^{-1} according to the hardness of the medium and temperature.

Saward and Topping[24] suggested that the growth of marine phytoplankton is inhibited by Cu concentrations between 17 and 30 μg l^{1}. In the present investigation, no inhibition on primary production was observed even when expected to 37.58 μg l^{-1} Cu. Zingmark[25] reported that Cd ion decreases photosynthetic rate at concentration as low as 10 μg l^{-1} in marine phytoplanktonic assemblages, which is almost same to the present findings of 12.11

$\mu g\ l^{-1}$ in brackishwater system. Filippis and Pallaghy[26] have reported inhibition of both GPP and R by heavy metals including mercury.

However, the variation in toxicity levels of trace metals may be due to the variation in phytoplankton population, species diversity and also chelating and buffering capacity of the water bodies which has also been opined by Jayaraj *et al.*[7] The toxicity of metals of algae is largely associated with their capacity to react with anzymes which may vary from habitat to habitat. Huntsman and Sunda[27] observed that some phytoplankton might be found living under a wide range of trace metal concentration by evolving various mechanisms for adaptation to growth at both high and low availability.

It can be inferred from the above observations that the pytoplankton production of Jagannath canal is not affected by the present ambient level of Cu, Zn, Pb and Cd and even they have the capacity to cope with the concentrations of these trace metals when increased by 5 $\mu g\ l^{-1}$. Further increase in metal levels, which may be added from the sewerage system of metropolis Calcutta, will be very severe for the canal ecosystem because of the inhibition of both production and respiration. This may induce anaerobic condition in the system. To mitigate the deterioration of Jagannath canal ecosystem due to metals, sewage of Calcutta city should be treated properly with the continuous monitoring of level of metals before release in the river.

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Effect of culture filtrates of some seed borne-fungi on seed germination and seedling growth of Sorghum

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Abstract

Effect of culture filtrates of *Colletotrichum gloeosporioides, Drechslera maydis, Fusarium oxyporum* and *Nigrospora oryzae* on seed germination and root and shoot elongation of two varieties (CSH-5 and local yellow) of sorghum was investigated. CSH-5 variety was more susceptible to the toxic effect of culture filtrates of these fungi than local yellow. *C. gloeosporioides* was more toxic in its effect, while *N. oryzae* was mild pathogen.

(**Keywords** : seed borne fungi/culture filtrate/growth/germination/sorghum)

Introduction

The fungi associated with seeds reduce the seed germination and seedling vigour. Vidyasekharan *et al.*[1] and Tripathi[2] have reported the inhibition of seed germination by metabolites of fungi studied by them. There are several reports of inhibition of seed germination, root and shoot elongation in different crops[3,4,5] but only meagre information is available on the effect of fungal culture filtrates on seed germination of Sorghum[6]. Hence in the present study the effect of culture filtrates of some of the common seed-borne fungi on seed germination, root and shoot elongation of Sorghum seed was undertaken with a view to assess the damage done to the seed germination and seedling growth.

Materials and Methods

Four of the dominant seed-borne fungi of sorghum (*Colletotrichum gloeosporioides, Drechslera maydis, Fusarium solani* and *Nigrospora oryzae*) were grown in Czapek's medium for 10, 20 and 30 days. Surface sterilized seeds were soaked in *D. maydis, F. oxysporum* and *N. oryzae* culture filtrate for an overnight (12 hrs.). The seeds thus treated were spread on blotter paper and moistened with respective culture filtrates for a week. Seeds soaked in sterilized water served as control. Roots and shoots of 3 days old seedlings were exercised and treated with the same culture filtrate for studying root and shoot

Table 1 – Effect of culture filtrates of different seed-borne fungi on seed germination, root and shoot elongation of two varieties of sorghum.

Name of the fungus	Age of culture filtrates (in days)	CSH-5			Local yellow		
		Germination inhibition (in %)	Root elongation (in cm)	Shoot elongation (in cm)	Germination inhibition (in %)	Root elongation (in cm)	Shoot elongation (in cm)
Colletotrichum gloeosporioides	10	49.55	76.84 ± 6.12	35.83 ± 10.26	45.47	80.57 ± 4.47	62.45 ± 7.06
	20	66.30	82.15 ± 5.22	70.83 ± 6.64	62.63	88.08 ± 4.20	64.90 ± 7.20
	30	79.48	86.83 ± 9.68	80.64 ± 10.65	70.46	71.81 ± 6.79	65.83 ± 11.78
Drechslera myadis	10	61.25	61.38 ± 8.33	79.06 ± 6.54	54.36	47.38 ± 6.10	77.37 ± 9.28
	20	70.38	63.38 ± 10.35	83.38 ± 6.54	66.55	49.88 ± 8.68	74.35 ± 9.20
	30	84.15	76.59 ± 7.56	79.37 ± 7.89	76.71	73.39 ± 5.89	88.80 ± 4.02
Fusarium solani	10	57.47	63.41 ± 7.42	57.17 ± 4.60	52.24	44.92 ± 11.39	59.78 ± 4.71
	20	65.23	81.90 ± 8.52	77.36 ± 9.28	59.45	71.36 ± 14.36	66.94 ± 9.71
	30	70.00	82.10 ± 7.07	88.70 ± 7.84	68.47	52.55 ± 7.46	76.91 ± 6.64
Nigrospora oryzae	10	52.12	37.86 ± 8.56	43.42 ± 5.50	43.90	43.61 ± 5.38	29.82 ± 6.08
	20	63.78	48.93 ± 8.96	52.18 ± 7.30	57.40	51.82 ± 5.88	38.88 ± 7.58
	30	69.28	58.55 ± 7.99	64.64 ± 7.13	63.45	62.75 ± 3.78	60.18 ± 5.40

elongation inhibition. At the end of seventh day, the percentage of seed germination inhibition, root and shoot elongation inhibition was calculated by the formula.

$$\text{Percentage of seed germination inhibition} = \frac{\text{Percentage of seed germination in treated}}{\text{Percentage of seed germination in control}} \times 100$$

Results and Discussion

Perusal of Table 1 reveals that the culture filtrates of different seed-borne fungi adversely affected the germination. *D. maydis* caused maximum seed germination inhibition, while *N. oryzae* caused the least. The toxicity of *C. gloeosporioides* and *F. solani* towards the sorghum seed germination was intermediate. Toxicity of the fungus increased with the increase in age of the fungus. CSH-5 was more susceptible than the local (yellow) variety.

Root elongation inhibition was maximum with *N. oryzae*. On the other hand, *F. solani, D. maydis* and *C. gloeosporioides* were almost equal in their inhibitory effect on shoot elongation of local (yellow) variety. The inhibitory effect of culture filtrates of *C. gloeosporioides* and *F. solani* decreased after 20 days of incubation, while reverse was true with *D. maydis* and *N. oryzae*. *D. maydis* exhibited a strong inhibitory effect on shoot elongation of local (yellow) variety. From the present investigations it is clear that the seed-borne fungi of sorghum not only acted adversely on the seed viability but also its growth.

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Effect of root-knot nematode, *Meloidogyne incognita* on essential oil contents of rose

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Abstract

The root-knot nematode, *Meloidogyne incognita* reduced significantly the photosynthetic leaf area, petal area, fresh as well as dry weight of flower and essential oil contents of rose when inoculated with higher inoculum levels. As the inoculum level increased, all the above parameters were decreased correspondingly. Being a sedentary endoparasitic in nature, this nematode might have influenced the nutritional food materials through blockage of the conducting tissues and affect the normal physiology of the plant. This seems to be one of the reasons for reducing the essential oil contents.

(**Keywords** : *Meloidogyne incognita*/rose/essential oil contents/inoculum level)

Introduction

Since time immemorial, rose has been loved and cultivated in gardens for ornamental purposes but now roses are grown on a commercial scale for perfumery industries, Ayurvedic and Unani medicines. The yield of oil from petals in India is far less than in developed countries. This could in part be because of climatic conditions, varietal differences and in part be because of losses due to pests and diseases.

Aligarh district of Uttar Pradesh is one of the important centers for rose cultivation under the name of Domask rose (*Rosa damascena* Mill.) which is similar to kazanlik rose of Bulgaria. About 400 hactares of land is dedicated in Aligarh district to the rose crop with an annual production of one metric ton of flowers per hactare. Though the roses are potential foreign exchange earner, the yield of rose oil is declining gradually. Besides other factors, plant-parasitic nematodes could be one of the reasons for this decline. Surprisingly no significant work has been done on this aspect so far. A few nematode genera (*Meloidogyne hapla, Xiphinema diversicaudatum* and *Helicotylenchus nannus*) has been found associated with the rose crop.[1,2] Saeed *et al.*[3] and Sudha and Koshy[4] have studied the association of plant-parasitic nematodes with roses. Goodey *et al.*[5] also observed that the root-knot nematode, *M. incognita* parasitized the rose plants.

In view of the economic importance of the rose crop, a survey was conducted to determine the presence of plant-parasitic nematodes in rose growing areas in Aligarh district. The following potential phytophagous nematodes have been identified : *Helicotylenchus indicus* Siddiqi, *Hoplolaimus indicus* Sher, *Pratylenchus coffeae* (Zimmerman) Filipjev & Schuurmans Stekhoven, *Xiphinema basiri* Siddiqi and *Hemicriconemoides mangiferae* Siddiqi. Other nematode species were found in less densities. Besides, the mild infestation of root-knot nematode causing root-galls in the root of rose plant was also observed. Examination of perineal pattern of female revealed the presence of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood. It was, therefore, considered worthwhile to study the role of *M. incognita* on photosynthetic leaf area, petal area, fresh and dry weight of flowers and essential oil contents of rose plant.

Materials and Methods

The studies were carried out during the year of 1997-1998 in glass-house condition of Department of Botany, Aligarh Muslim University, Aligarh. Cutting of Domask rose (*Rosa damascena* Mill.), 22.5 cm in length, were taken from the best of healthy old bushes. The woody cutting were planted in 30 cm diameter earthern pots containing 4 kg steam sterilized soil (clay, sand and compost at the ratio of 7:2:1) during the month of October. One month after planting, when plants were established and had begun to show root-growth, inoculation was made with 2nd stage juveniles of root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood at the rates of 5000, 10000, 15000 and 20000 per pot. One set was uninoculated and served as control. Each treatment including the uninoculated control was replicated five times.

Observations were made after four months of inoculation. When plants showed sufficient flowering, Photosynthetic leaf area and petal area were measured with the help of planimeter, fresh as well as dry weight of flower was also taken. Number of root-galls were counted. Extraction of essential oil contents were measured with the help of Clevenger apparatus[6]. Rose petals were collected in early morning and placed in a distilling flask before sufficient water added and then connected with a condenser. The regulated heating of flask was adjusted with a mentle heater. The water vapours containing drops of essential oil were settled and flouted above the surface of water which were then collected and weighed.

Results and Discussion

The results presented in Table 1 clearly indicate that the root-knot nematode, *M. incognita*, reproduced on rose plant, as is evident from the presence of root-galls, and

Table 1 – Effect of different inoculum level of root-knot nematode, *Meloidogyne incognita* on photosynthetic leaf area, petal area, fresh and dry weight of flower and essential oil contents of Domask rose (*Rosa damascena* Mill.)*.

Inoculum Level	Photo-synth-etic leaf area (cm^2)[1]	%reduc-tion over control	Petal area (cm^2)[1]	%reduc-tion over control	Fresh weight of flower (g)	%reduc-tion over control	Dry weight of flower (g)	%reduc-tion over control	% oil content	%reduc-tion over control	Number of root-galls per plant
Uninoculated	11.25	–	15.50	–	6.78	–	0.57	–	0.045	–	–
5,000	9.75	13.33	14.50	4.91	5.10	11.76	0.55	3.50	0.042	6.20	67.5
10,000	8.00	28.89	13.25	13.11	4.60	20.41	0.53	7.01	0.040	9.78	118.7
15,000	7.25	35.55	11.25	26.22	4.05	29.93	0.53	7.01	0.036	18.55	158.3
20,000	6.00	46.66	9.75	36.66	3.45	40.31	0.51	10.52	0.030	33.35	194.5
C.D. (P = 0.05)	0.78		1.33		0.54		0.036		0.004		38.63
C.D. (P = 0.01)	1.07		1.83		0.74		0.050		0.006		53.23

*Data represents mean of five replicates.
1 = Mean of 10 observation of each treatment.

caused considerable damage in all parameters. As the inoculum level increased, the photosynthetic leaf area, petal area, fresh as well as dry weight of flower and essential oil contents were decreased. The significant reduction was observed in photosynthetic leaf area and fresh weight of flowers when plants were inoculated with 5000 or more nematodes, whereas the petal areas, dry weight of flower and essential oil contents were reduced significantly in those plants inoculated with 10000 or more nematodes. The lowest percent reduction in oil contents was noted as 6.20% with 5000 nematodes whereas the highest being reported as 33.35% with 20000 nematodes inoculated per plant. The highest number of root-galls were noted as 194.5 in those plants inoculated with 20000 nematode per plant. As the literature revealed, the root-knot nematode, *M. incognita,* being a sedentary endoparasitic in nature caused enormous damage to the plant through vascular system where its female settled after infection[7,8,9] leading to various abnormal changes in the host-physiology[10,11,12]. This prevented the nutritional food materials to some extent to reach the top of the plant and resulted in reduction of total photosynthetic leaf area, which might have affected petal area too. Several reports are available that the root-knot nematode influenced the normal supply of nutritional food materials of *Phaseolus aureus*[13] and reduced the photosynthetic pigment in leaves of tomato[14,15]. Similar reports have been confirmed by Ahmad *et al.*[16] and Tiyagi and Alam[17] where movement of food materials was restricted from reaching the fertile branches due to infection by root-knot, reniform and stunt nematodes. All these factors may be contributed towards the reduction in essential oil contents.

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Analysis of natural vegetation of an abandoned open cast coal mine in monsoon climate of Eastern India

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Abstract

The quantitative analysis of the natural vegetation on overburdens of an abandoned unreclaimed coal mine in South Bolanda, Talcher, Orissa revealed that the plant communities occurring on coal mine wastes after 30 years of abandonment represent early stages of a slow primary succession and also that the succession has proceeded predominantly by species enrichment over the years, as evinced by the general sparseness of plant and low plant cover.

(Keywors: flora/vegetation/community analysis/coal mine overburdens)

Introduction

Surface mining inevitably produces major environmental disturbances since vegetation, top soil and underlying soil mantle have to be removed to gain access to the minerals beneath[1]. Not only is there local destruction of the ecosystem and loss of productive areas, but there can also be serious pollution problems arising from wind and water borne materials. The problem can be further aggravated as natural watercourses may be disturbed due to disruption of surface and ground water circulation. Various living communities may also be affected. In mined lands, certain extreme soil conditions may prevent plant growth, particularly physical conditions and lack of certain nutrients and toxicity[2].

In India, more than 80,000 ha of land are under various kinds of stress due to mining activities[3] and much larger area is disturbed by other associated activities. As demands for minerals grow, increasing area of land will be affected in the future years. There is the great need to find ways in which either the original or substitute vegetation can be reinstated quickly and economically. The vegetation must rapidly become self-sustaining so that further harmful impacts after the initial treatments are minimal. It should also restore fertility of the degraded materials on which it is established[1,4].

In order to take up revegation programme of mine wastes it is essential to understand the composition of the natural vegetation, their diversity and distribution. Acidity, poor

water holding capacity and inadequate supply of nutrients are the major limiting factors for establishment of vegetation cover on coal mine spoils[5].

The South Bolanda colliery, the largest open cast coal mines which mostly produces bitiminous types of coal, is situated in the valley of river Brahmani at Talcher (Angul District), Orissa about 88 kilometers north-west of Cuttack. It lies between 20° 29'N and 21°42'N latitudes and 84°16'E and 86°20'E longitudes and is 564m above MSL. The area is predominantly an undulating terrain. A gravel ridge forms the northern boundary of the flock. Rock exposures are limited to few sand and stone and pebble beds. The coal bearing area is represented by medium to coarse-grained pale brownish yellow, loose-textured sandstone with thin shale bands and coal seams. The geological structure of South Bolanda Block is a part of a shallow basin. The thinning of the seam towards outcrop and its ultimate pinching out is a characteristic feature of area. Coal mine overburdens dumped for over 30 years have resulted in formation of huge dumps almost like hillocks stretching over several hectares of land with very poor vegetation cover. An estimated 3.5 hectares of land has been disturbed by this coal mining and related activities.

Summer is very hot and the temperature rises to as high as 49°C in May/June with high humidity. The mean annual rainfall ranges from 100 cm to 150 cm. Monsoon breaks towards the end of June and continues upto the middle of October. In addition to the above rainfall, occasional heavy showers are also received due to the receding North-East monsoon in the month of November and December.

In the present study the natural vegetation occuring on coal mine wastes of South Bolanda, Talcher has been studied through standard ecological methods.

Materials and Methods

The nested quadrat method (1m x 1m) was used to sample the natural vegetation of the coal mine dumps at South Bolanda during the period August-November, 1987. Thirty seven quadrats were taken as randomly as possible from base, mid slope and crest positions of seven overburden dumps to minimize errors. Data were analyzed for absolute frequency, relative frequency, absolute density, relative density and importance value. The formulae based on Brower and Zar[6] as described below were followed.

$$\textit{Absolute frequency} = \frac{\textit{frequency of a species}}{\textit{total frequency of all species}} \times 100$$

$$\textit{Absolute density} = \frac{\textit{number of individuals of a species}}{\textit{total area sampled } (37m^2)}$$

$$Relative\ density = \frac{density\ of\ a\ species}{density\ of\ all\ species} \times 100$$

Importance value = Relative frequency + Relative density

Collected plant samples were identified and preserved as herbarium specimens in the Herbarium of Regional Plant Resource Centre, Bhubaneswar. Correct botanical names were ascertained to each of them in accordance with the rules of the International Code of Botanical Nomenclature.

Results and Discussion

The study revealed the occurrence of 141 species of trees, shrubs and herbs belonging to 115 genera under 44 families on coal mine overburdens (Table 1). Tree species like *Azadirachta indica, Ziziphus mauritiana, Ailanthus exelsa, Dalbergia sissoo, Tamarindus indica, Acacia leucophloea, Haldina cordifolia, Mitragyna parvifolia, Morinda coreia, Diospyros melanoxylon, D. cordifolia, Symplocos racemosa, Alstonia scholaris, Lagerstroemia parviflora, Holarrhena pubescens, Strychnos nux-vomica, Trema orientalis, Ficus benghalensis, F. religiosa* etc. predominant in the adjoining forests were found to have established on the mine wastes.

Table 1 – List of plants occurring on coal mine overburdens at South Bolanda.

1. *Acacia leucopholea* (Roxb.) Willd. (1878)
2. *Acanthospermum hispidum* DC. (1879)
3. *Achyranthus aspera* L. (1833)
4. *Adenosma indianum* (Lour.) Merr. (1880)
5. *Adiantuim philippense* L. (1881)
6. *Aeschynomene indica* L. (1899)
7. *Ageratum conyzoides* L. (1888)
8. *Ailanthus excelsa* Roxb. (1889)
9. *Alstonia scholaris* (L.) R.Br. (1891)
10. *Alternanthera sessilis* (L.) R.Br. ex. DC. (1837)
11. *Alysicarpus rogosus* (Willd.) DC. (1875)
12. *Alysicarpus vaginalis* DC. (1822)
13. *Ammannia baccifera* L. (1895)
14. *Andrographis paniculata* (Bum.f.) Wall. ex. Nees (1813)
15. *Annoa squamosa* L. (1887)
16. *Antidesma ghaesembilla* Gaertn. (1882)
17. *Aristida setacea* Retz. (1842)
18. *Atylosia scarabaeoides* (L.) Benth. (1810)
19. *Azadirachta indica* A. Juss. (1885)
20. *Blumea alata* (D. Don) DC. (1896)
21. *Blumea obliqua* (L.) Druce (1883)
22. *Blumeopsis flava* (DC.) Gangnep. (1886)
23. *Bombax campestris* L. (1884)
24. *Brasica campestris* L. (1884)
25. *Breynia retusa* (Dennst.) Alston. (3882)
26. *Bulbostylis barbata* (L.) R.Br. (1890)
27. *Calotropis gigantea* (L.) R.Br. (1890)
28. *Canscora decussata* Schult. & Schult.f. (1898)
29. *Canscora diffusa* (Vahl) R.Br. (1897)
30. *Casearia elliptica* Willd. (3833)
31. *Casearia graveolens* Dalz. (3834)
32. *Cassytha filiformis* L. (3859)
33. *Catharanthus roseus* (L.) G.Don. (1847)
34. *Celosia argentea* L. (1860)
35. *Centella asiatica* (L.) Urb. (3862)
36. *Chloris barbata* (L.) Sw. (1805)

37. *Chromalaena odorata* (Linn.) King & Robinson (1824)
38. *Cleome ciscosa* L. (1854)
39. *Commelina kurzii* C.B. Clarke (1892)
40. *Commelina obliqua* Buch-Ham. (3863)
41. *Crotolaria prostrata* Rotti. (3881)
42. *Croton bonplandianum* Baill. (1894)
43. *Cynodon dactylon* (L.) Pers. (1893)
44. *Cyperus castaneus* Willd. (3861)
45. *Cyperus compressus* L. (3880)
46. *Cyperus iria* L. (3860)
47. *Dactylocenium aegyptium* (L.) P. Beauv. (1840)
48. *Dalbergia sissoo* Roxb. (3835)
49. *Desmodium triflorum* (L.) DC. (1819)
50. *Dichanthium bladhii* (Retz.) Clayon (1832)
51. *Dichanthium pertusum* (L.) Clayton (1841)
52. *Digitaria ciliaris* (Retz.) Koeler. (1862)
53. *Digitaria longiflora* (Retz.) Pers. (3843)
54. *Diospyros chloroxylon* Roxb. (3848)
55. *Diospyrs cordifolia* Roxb. (3864)
56. *Disopyros melamnoxylon* Roxb. (3865)
57. *Echinochola colona* (L.) Link. (3883)
58. *Echinochola stagnina* (Retz.) P. Beauc. (3884)
59. *Eclipta prostrata* (L.) (3840)
60. *Elusine indica* (L.) Gaertn. (1858)
61. *Emilia sonchifolia* (L.) DC. (3842)
62. *Eragrostis ciliaris* (L.) R.Br. (1835)
63. *Eragrstis gagentica* (Roxb.) Steud. (3866)
64. *Eragrostis uniloides* (Retz.) Nees ex Steud. (3893)
65. *Ernthemum capens* L. (3839)
66. *Euphorbia hirta* L. (1861)
67. *Euphorbia thymifolia* L. (3847)
68. *Evolvulus alsinoides* (L.) Linn. (3892)
69. *Evolvulus nummularius* (L.) L. (1821)
70. *Ficus benghalensis* L. (3837)
71. *Ficus religiosa* L. (3885)
72. *Fimbristylis acuminata* Vahl (1874)
73. *Fimbristylis aestivalis* (Retz.) Vahl (3851)
74. *Flacourtia indica* (Burm.f.) Merr. (3886)
75. *Gomphrena celosioides* Mart. (3846)
76. *Gymnema sylvestre* (Retz.) R.Br. ex Schult. (3887)
77. *Haldina cordifolia* (Roxb.) Ridsd. (3894)
78. *Hedytis corymbosa* (L.) Lam. (1849)
79. *Hedyotis erecta* Manilal & Sivarajan (3895)
80. *Hedyotis herbacea* L. (3845)
81. *Hemidesmus indicus* (L.) R.Br. (1806)
82. *Heteropogon contortus* (L.) P. Beauv. ex Roem & Schult (1838)
83. *Holarrhena pubescens* (Buch. Ham.) Wall. ex G.Don. (5746)
84. *Hybanthus enneasperimus* (L.) F.V. Muell. (3850)
85. *Hygrophila polysperma* T. Anders. (3888)
86. *Hyptis suaveolens* (L.) Poit. (1801)
87. *Ichnocarpus frutescens* (L.) R.Br. (3896)
88. *Indigofera astragalina* DC. (3836)
89. *Indigofera linnaei* Ali (3844)
90. *Ipomea carnea* Jacq. (3889)
91. *Ixora arborea* Roxb. ex Sm. (3838)
92. *Justicia diffusa* Willd. (3897)
93. *Kyllinga triceps* Rottb. (3841)
94. *Laqerstroemia parvi* Roxb. (5831)
95. *Laggera alata* (D.Don) DC. (1883)
96. *Lantana camara* L. var. aculeata (Linn.) Moldenke. (3898)
97. *Leptadenia reticulata* (Retz.) Wt. & Arn. (3891)
98. *Leucas aspera* (Willd.) Link. (3890)
99. *Leucas indica* (L.) R.Br. ex Vatke (3832)
100. *Lindernia parviflora* (Roxb.) Haines (3849)
101. *Ludwigia perennis* L. (1859)
102. *Lygodium flexuosum* (L.) Sw. (3852)
103. *Mariscus paniceus* (Rottb.) Vahl. (3871)
104. *Mitracarpus villosus* (Sw.) DC. (5751)
105. *Mollugo pentphylla* L. (3872)
106. *Morinda coreia* Buch.-Ham. (3877)
107. *Mitragyna parvifolia* (Roxb.) Korth. (5745)
108. *Ocimum basilicum* L. (1848)
109. *Oligochaea ramos* (Roxb.) Wagen. (5747)
110. *Paspalum scrobiculatum* L. (1807)
111. *Pennisetumk pedicellatum* Trin. (1823)
112. *Phyllanthus amarus* Schumach. & Thonn. (3873)
113. *Phyllanthus reticulatus* Poir. (3830)

114. *Phyllanthus virgatus* Forst. f. (1843)
115. *Psidium guajava* L. (3854)
116. *Saccharum spontaneum* L. (3856)
117. *Sacciolepsis indica* (L.) Chase. (3876)
118. *Scoparia dulcis* L. (5740)
119. *Sebastiania chamelea* (Linn.) Muell. (5744)
120. *Sida cordata* (Bum. f.) Borssum (3869)
121. *Solanum virginianum* L. (3870)
122. Spermacoce articlaris L.f.(5743)
123. *Spermacoce pusilla* Wall. (5741)
124. *Sphenoclea zeylancia* Gaetrtn. (3879)
125. *Strychnos nux-vomica* L. (3855)
126. *Symplocos racemsa* Roxb. (5742)
127. *Tamarindus indica* L. (3867)
128. *Tephrosia purpurea* (L.) Pers. (3857)
129. *Tragia involucrata* L. (1920)
130. *Trema orientalis* (L.) Bl. (3875)
131. *Tridax procmbens* L. (1826)
132. *Triumfetta neglecta* Wt. & Arn. (1852)
133. *Typha angustata* Bory & Chaub. (3874)
134. *Urena lobata* L. (1812)
135. *Urochloa panicoides* P. Beauv. (1855)
136. *Vernonia cinerea* (L.) Less. (1838)
137. *Woodfordia fruticosa* (L.) Kurz. (3878)
138. *Xanthium indicum* Koenig. (3868)
139. *Ziziphus mauritina* Lam. (1827)
140. *Ziziphus oenoplia* Mill. (1816)
141. Zornia gibbosa Span. (3853)

N,B, = Field number for each species has been cited in parenthesis after author citation.

Sixty nine species belonging to 61 genera and 21 families of herbs and small shrubs were recorded within the 37 nested quadrats (Table 2). Of these, 41 taxa belonged to the families Poaceae, Asteraceae, Euphorbiaceae, Fabaceae and Rubiaceae. The herbs with a combined relative frequency of more than 38.6 percent belonged to *Tridax procumbens, Eragrostis ciliaris, Croton bonplandianum, Desmodium triflorum, Mollugo pentaphylla, Hedyotis corymbosa, Saccharum spentaneum* and *Spermacoce articularis. Desmodium triflorum* and *Tridax procumbens* were the two dominant herbaceous taxa with importance value of 18.268 and 17.966 respectively. Herbs like *Desmodium trflorum, Tridax procumbens, Spermacoce articularis, Eragrostis ciliaris, Hedyotis corymbosa, Saccharum spontaneum* and *Croton bonplandiamum* had the importance value of more than 88.74%. Total plant cover and species richness varied significantly with slope positions in the mine overburdens. They had their highest frequency at the base of the slope and lowest at the crest position. Further, it was observed that most of the plants colonizing the mined land are local and have migrated from adjacent forest areas and scrub jungles over the period of time.

Floral distribution through quadrat analysis provided significant clues for revegetation process. The quadrat analysis based on absolute frequency, relative frequency, absolute density and relative density of the different plant species in the area understudy following the formulae of Brower and Zar[6] revealed that in South Bolanda coal mine, the herbs like *Tridax procumbens, Eragrostis ciliaris, Croton bonplandianum, Desmodium triflorum, Mollugo pentaphylla, Hedytis corymbosa, Saccharum spontaneum* and *Spermacoce articularis* grows with a combined relative frequency of more than 38.6 per cent. *Desmodium triflorum* and *Tridax procummens* were the species with relative frequency of 4.960 and

Table II. Analysis of natural vegetation on coal mine overburdens

Name of the species	Absolute frequency	Relative frequency	Absolute density	Relative density	Importance value
Acanthospermum hispidum	5	1 305	0.0138	0.7598	2.0648
Adenosma indianum	4	1.044	0.0109	0.6001	1.6441
Adiantum philippense	1	0.261	0.0014	0.0770	0.2380
Alternanthera sessilis	2	0.522	0.0036	0.1982	0.7202
Alysicarpus rugosus	1	0.261	0.0051	0.2808	0.5418
Alysicarpus vaginalis	10	2.610	0.0445	2.4501	5.0601
Andrographis paniculata	6	1.566	0.0182	1.0020	2.5680
Aristida setacea	10	2.610	0.0357	1.9656	4.5756
Atylosia scarabaeoides	8	2.088	0.0219	1.2058	3.2938
Blumea alata	4	1.044	0.0080	0.4404	1.4844
Blumea obliqua	9	2.349	0.0277	1.5251	3.8741
Blumeopsis flava	3	1.783	0.0043	0.2367	1.0197
Bulbostylis barbata	2	0.522	0.0080	0 4404	0.9624
Calotropis gigantea	4	1.044	0.0065	0 3578	1.4018
Canscora decussata	1	0.261	0.0014	0.0770	0.3380
Catharanthus roseus	2	0.522	0.0080	0.4404	0.9620
Celosia argentea	1	0.261	0.0014	0.0770	0.3380
Chromolena odorata	12	3.133	0.0540	2.3732	5.5062
Commelina obliqua	1	0.261	0.0007	0.0385	0.2995
Crotalaria prostrata	1	0.261	0.0014	0.0770	0.3380
Croton bonplandianum	19	4.960	0.0547	3.0117	7 9717
Cynodon dactylon	12	3.133	0.0664	3.6559	6.7889
Cyperus compressus	2	0.522	0.0021	0.1156	0.6376
Dactyloctenium aegyptium	2	0.522	0.0102	0.5616	1.0836
Desmodium trifolium	19	4.960	0.2417	13.3080	18.2680
Dichanthium pertusum	1	0.261	0.0007	0.0385	0.2995
Digitaria ciliaris	4	1.044	0.0109	0.6001	1.6441
Digitaria longiflora	2	0.522	0.0036	0.1982	0.7202
Echinochloa colona	1	0.261	0.0043	0.2367	0.4977
Eclipta prostrata	4	1.044	0.0080	0.4404	1.4844
Emilia sonchifolia	12	3.133	0.0511	2.8135	.5.9465
Eragrostis ciliaris	23	6.005	0.1022	5.6271	11.6321
Eragrostis uniloides	2	0.522	0.0043	0.2367	0.7587
Eranthemum capens	1	0.261	0.0021	0.1156	0.3766
Euphorbia hirta	2	0.522	0.0021	0 1156	0.6376
Euphorbia thymifolia	2	0.522	0.0057	0.3138	0.8358
Evolvulus alsinoides	10	2.610	0.0255	1.4040	4 0140
Evolvulus nummularius	10	2.610	0.0496	2.7309	5.3409
Hedyotis corymbosa	17	4.438	0.1029	5.6656	10.1036
Hedyotis herbacea	2	0.522	0.0255	1.4040	1.9260
Heteropogon contortus	6	1.566	0.0255	1.4040	2.9700
Hybanthus enneaspermus	1	0.261	0.0007	0.0385	0 2995
Hyptis suaveolens	8	2.088	0.0401	2.2079	4.2959
Ipomoea carnea	1	0.261	0.0007	0.0385	0.2995
Kyllinga triceps	1	0.261	0.0007	0.0385	0.4206
Leptadenia reticulata	2	0.522	0.0014	0.0770	0.5990
Leucas aspera	1	0.261	0.0029	0.1596	0.4206
Lindernia parviflora	1	0.261	0.0014	0.0770	0.3380
Mitracarpus villosus	6	1.566	0.0153	0.5424	2.4084
Mollugo pentaphylla	17	4.438	0.0511	2.8135	7.2515
Ocimum basilicum	1	0.261	0.0043	0.2367	0.4977
Paspalum scrobiculatum	4	1.044	0.0980	0.7212	1.4844
Pennisetum pedicellatum	5	1.305	0.0189	1.0406	2.3456

Phyllanthus amarus	7	1.827	0.0109	0.6001	2.4371
Phyllanthus virgatus	4	1.044	0.0131	0.7212	1.7654
Saccharum spontaneum	17	4.438	0.0701	3.8597	8.2977
Scoparia dulcis	2	0.522	0.0255	1.4040	1.9260
Sebastiania chamaelea	8	2.088	0.0182	1.0020	3.0900
Sida cordata	2	0.522	0.0065	0.3578	0.8798
Solanum virginianum	4	1.044	0.0094	0.5176	1.5616
Spermacoce articularis	14	3.655	0.1789	9.8502	13.5052
Tragia involucrata	1	0.261	0.0029	0.1597	0.4207
Tridax procumbens	24	6.266	0.2125	11.7003	17.9663
Triumfetta neglecta	1	0.261	0.0007	0.0385	0.2995
Urena lobata	1	0.261	0.0007	0.0385	0.2995
Urochloa panicoides	4	1.044	0.0350	1.9271	1.9711
Vernonia cinerea	2	0.522	0.0021	0.1156	0.6376
Xanthium indicum	2	0.522	0.0065	0.3579	0.8799
Zornia gibbosa	2	0.522	0.0021	0.1156	0.6376
	383	99.976	1.8162	99.368	199.344

6.266 respectively. Most of these herbs are indicators of early stages of plant colonization and the primary stage of plant succession in barren lands. These observations are in confirmity with Smyth[7] who reported early succession patterns with a native species seed mix on amended and unamended coal mine spoil in the Rocky Mountains of South eastern British Columbia, Canada. Several workers[8-12] have also made similar observations on plant succession in coal surface-mined areas.

Being a 30 year old undisturbed overburden, South Bolanda colliery site provided an excellent opportunity for the study and analysis of the natural plant communities on coal surface mined lands. The natural vegetation represents aceral successional stage towards the regional vegetation progressing at a very slow pace. The richness of the species diversity but general sparsenss of plants is suggestive of the fact that succession has proceeded predominantly by enrichment with local taxa over a period of time. This community has developed mainly from native species spreading from contiguous and nearby forest scrub jungles and wastelands. In general, it could be observed that at the basal portion of the mine overburden dumps support the growth and development of large number of native plant species. Analysis of the diversity, distribution and abundance of flora resulting from natural plant succession will be of help in selection of suitable plant species for successful revegetation of surface mined lands, especially for forestry and use as wild life habitats.

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